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(54) Title: **CONJUGATES USEFUL IN THE TREATMENT OF PROSTATE CANCER**

(57) Abstract

Chemical conjugates which comprise oligopeptides, having amino acid sequences that are selectively proteolytically cleaved by free prostate specific antigen (PSA) and known cytotoxic agents are disclosed. Such conjugates are useful in the treatment of prostatic cancer and benign prostatic hypertrophy (BPH).

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TITLE OF THE INVENTIONCONJUGATES USEFUL IN THE TREATMENT OF PROSTATE
CANCER5 BACKGROUND OF THE INVENTION

In 1994 cancer of the prostate gland is expected to be diagnosed in 200,000 men in the U.S. and 38,000 American males will die from this disease (Garnick, M.B. (1994). The Dilemmas of Prostate Cancer. Scientific American, April:72-81). Thus, prostate cancer is the
10 most frequently diagnosed malignancy (other than that of the skin) in U.S. men and the second leading cause of cancer-related deaths (behind lung cancer) in that group.

Prostate specific Antigen (PSA) is a single chain 33 kDa glycoprotein that is produced almost exclusively by the human
15 prostate epithelium and occurs at levels of 0.5 to 2.0 mg/ml in human seminal fluid (Nadji, M., Taber, S.Z., Castro, A., et al. (1981) Cancer 48:1229; Papsidero, L., Kuriyama, M., Wang, M., et al. (1981). JNCI 66:37; Qui, S.D., Young, C.Y.F., Bihartz, D.L., et al. (1990), J. Urol. 144:1550; Wang, M.C., Valenzuela, L.A., Murphy, G.P., et al. (1979).
20 Invest. Urol. 17:159). The single carbohydrate unit is attached at asparagine residue number 45 and accounts for 2 to 3 kDa of the total molecular mass. PSA is a protease with chymotrypsin-like specificity (Christensson, A., Laurell, C.B., Lilja, H. (1990). Eur. J. Biochem. 194:755-763). It has been shown that PSA is mainly responsible for
25 dissolution of the gel structure formed at ejaculation by proteolysis of the major proteins in the sperm entrapping gel, Semenogelin I and Semenogelin II, and fibronectin (Lilja, H. (1985). J. Clin. Invest. 76:1899; Lilja, H., Oldbring, J., Rannevik, G., et al. (1987). J. Clin. Invest. 80:281; McGee, R.S., Herr, J.C. (1988). Biol. Reprod. 39:499).
30 The PSA mediated proteolysis of the gel-forming proteins generates several soluble Semenogelin I and Semenogelin II fragments and soluble fibronectin fragments with liquefaction of the ejaculate and release of progressively motile spermatozoa (Lilja, H., Laurell, C.B. (1984). Scand. J. Clin. Lab. Invest. 44:447; McGee, R.S., Herr, J.C. (1987).

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Biol. Reprod. 37:431). Furthermore, PSA may proteolytically degrade IGFBP-3 (insulin-like growth factor binding protein 3) allowing IGF to stimulate specifically the growth of PSA secreting cells (Cohen et al., (1992) J. Clin. Endo. & Meta. 75:1046-1053).

- 5 PSA complexed to alpha 1 - antichymotrypsin is the predominant molecular form of serum PSA and may account for up to 95% of the detected serum PSA (Christensson, A., Björk, T., Nilsson, O., et al. (1993). J. Urol. 150:100-105; Lilja, H., Christensson, A., Dahlén, U. (1991). Clin. Chem. 37:1618-1625;
- 10 Stenman, U.H., Leinoven, J., Alfthan, H., et al. (1991). Cancer Res. 51:222-226). The prostatic tissue (normal, benign hyperplastic, or malignant tissue) is implicated to predominantly release the mature, enzymatically active form of PSA, as this form is required for complex formation with alpha 1 - antichymotrypsin (Mast, A.E., Enghild, J.J.,
- 15 Pizzo, S.V., et al. (1991). Biochemistry 30:1723-1730; Perlmutter, D.H., Glover, G.I., Rivetna, M., et al. (1990). Proc. Natl. Acad. Sci. USA 87:3753-3757). Therefore, in the microenvironment of prostatic PSA secreting cells the PSA is believed to be processed and secreted in its mature enzymatically active form not complexed to any
- 20 inhibitory molecule. PSA also forms stable complexes with alpha 2 - macroglobulin, but as this results in encapsulation of PSA and complete loss of the PSA epitopes, the in vivo significance of this complex formation is unclear. A free, noncomplexed form of PSA constitutes a minor fraction of the serum PSA (Christensson, A., Björk, T., Nilsson, O.,
- 25 et al. (1993). J. Urol. 150:100-105; Lilja, H., Christensson, A., Dahlén, U. (1991). Clin. Chem. 37:1618-1625). The size of this form of serum PSA is similar to that of PSA in seminal fluid (Lilja, H., Christensson, A., Dahlén, U. (1991). Clin. Chem. 37:1618-1625) but it is yet unknown as to whether the free form of serum PSA may
- 30 be a zymogen; an internally cleaved, inactive form of mature PSA; or PSA manifesting enzyme activity. However, it seems unlikely that the free form of serum PSA manifests enzyme activity, since there is considerable (100 to 1000 fold) molar excess of both unreacted alpha

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- 1 - antichymotrypsin and alpha 2 - macroglobulin in serum as compared with the detected serum levels of the free 33 kDa form of PSA (Christensson, A., Björk, T., Nilsson, O., et al. (1993). J. Urol. 150:100-105; Lilja, H., Christensson, A., Dahlén, U. (1991). Clin. Chem. 37:1618-1625).

- Serum measurements of PSA are useful for monitoring the treatment of adenocarcinoma of the prostate (Duffy, M.S. (1989). Ann. Clin. Biochem. 26:379-387; Brawer, M.K. and Lange, P.H. (1989). Urol. Suppl. 5:11-16; Hara, M. and Kimura, H. (1989). J. Lab. Clin. Med. 113:541-548), although above normal serum concentrations of PSA have also been reported in benign prostatic hyperplasia and subsequent to surgical trauma of the prostate (Lilja, H., Christensson, A., Dahlén, U. (1991). Clin. Chem. 37:1618-1625). Prostate metastases are also known to secrete immunologically reactive PSA since serum PSA is detectable at high levels in prostatectomized patients showing widespread metastatic prostate cancer (Ford, T.F., Butcher, D.N., Masters, R.W., et al. (1985). Brit. J. Urology 57:50-55). Therefore, a cytotoxic compound that could be activated by the proteolytic activity of PSA should be prostate cell specific as well as specific for PSA secreting prostate metastases.

- It is the object of this invention to provide a novel anti-cancer composition useful for the treatment of prostate cancer which comprises oligopeptides, that are selectively proteolytically cleaved by free prostate specific antigen (PSA) and that include a cyclic amino acid having a hydrophilic substituent, in conjugation with a cytotoxic agent.

Another object of this invention is to provide a method of treating prostate cancer which comprises administration of the novel anti-cancer composition.

30 SUMMARY OF THE INVENTION

Chemical conjugates which comprise oligopeptides, having amino acid sequences that are selectively proteolytically cleaved by free prostate specific antigen (PSA) and that include a cyclic amino acid having a hydrophilic substituent, and known cytotoxic agents are

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disclosed. Such conjugates are useful in the treatment of prostatic cancer and benign prostatic hyperplasia (BPH).

DETAILED DESCRIPTION OF THE INVENTION

5 The instant invention relates to novel anti-cancer compositions useful for the treatment of prostate cancer. Such compositions comprise the oligopeptides covalently bonded directly, or through a chemical linker, to a cytotoxic agent. The oligopeptides are chosen from oligomers that are selectively recognized by the free
10 prostate specific antigen (PSA) and are capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen. Such a combination of an oligopeptide and cytotoxic agent may be termed a conjugate.

 The conjugates of the instant invention are further
15 characterized by incorporation of a cyclic amino acid having a hydrophilic substituent as part of the oligopeptides, said cyclic amino acid which contributes to the aqueous solubility of the conjugate. Examples of such hydrophilic cyclic amino acids include but are not limited to hydroxylated, polyhydroxylated and alkoxylated proline and
20 pipecolic acid moieties.

 Ideally, the cytotoxic activity of the cytotoxic agent is greatly reduced or absent when the oligopeptide containing the PSA proteolytic cleavage site is bonded directly, or through a chemical linker, to the cytotoxic agent and is intact. Also ideally, the cytotoxic
25 activity of the cytotoxic agent increases significantly or returns to the activity of the unmodified cytotoxic agent upon proteolytic cleavage of the attached oligopeptide at the cleavage site.

 Furthermore, it is preferred that the oligopeptide is selected from oligopeptides that are not cleaved or are cleaved at a much slower
30 rate in the presence of non-PSA proteolytic enzymes when compared to the cleavage of the oligopeptides in the presence of free enzymatically active PSA.

 For the reasons above, it is desirable for the oligopeptide to comprise a short peptide sequence, preferably less than ten amino
35 acids. Most preferably the oligopeptide comprises seven or six amino

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acids. Because the conjugate preferably comprises a short amino acid sequence, the solubility of the conjugate may be influenced to a greater extent by the generally hydrophobic character of the cytotoxic agent component. Therefore, the hydrophilic substituents on the cyclic amino
5 acid of the instant conjugates are selected to offset or diminish such a hydrophobic contribution by the cytotoxic agent.

While it is not necessary for practicing this aspect of the invention, a preferred embodiment of this invention is a conjugate wherein the oligopeptide, and the chemical linker if present, are
10 detached from the cytotoxic agent by the proteolytic activity of the free PSA and any other native proteolytic enzymes present in the tissue proximity, thereby presenting the cytotoxic agent, or a cytotoxic agent that retains part of the oligopeptide/linker unit but remains cytotoxic, into the physiological environment at the place of proteolytic cleavage.
15 Pharmaceutically acceptable salts of the conjugates are also included.

It is understood that the oligopeptide that is conjugated to the cytotoxic agent, whether through a direct covalent bond or through a chemical linker, does not need to be the oligopeptide that has the greatest recognition by free PSA and is most readily proteolytically
20 cleaved by free PSA. Thus, the oligopeptide that is selected for incorporation in such an anti-cancer composition will be chosen both for its selective, proteolytic cleavage by free PSA and for the cytotoxic activity of the cytotoxic agent-proteolytic residue conjugate (or, in what is felt to be an ideal situation, the unmodified cytotoxic agent) which
25 results from such a cleavage. The term "selective" as used in connection with the proteolytic PSA cleavage means a greater rate of cleavage of an oligopeptide component of the instant invention by free PSA relative to cleavage of an oligopeptide which comprises a random sequence of amino acids. Therefore, the oligopeptide component of the instant
30 invention is a preferred substrate of free PSA. The term "selective" also indicates that the oligopeptide is proteolytically cleaved by free PSA between two specific amino acids in the oligopeptide.

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The oligopeptide components of the instant invention are selectively recognized by the free prostate specific antigen (PSA) and are capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen. Such oligopeptides comprise an
5 oligomer selected from:

- a) HaaXaaSerTyrGlnSerSer (SEQ.ID.NO.: 1);
- b) HaaTyrGlnSerSer (SEQ.ID.NO.: 2);
- 10 c) HaaXaaLysTyrGlnSerSer (SEQ.ID.NO.: 3);
- d) HaaXaaLysTyrGlnSerSer (SEQ.ID.NO.: 4);
- 15 e) HaaXaahArgTyrGlnSerSer (SEQ.ID.NO.: 5);
- f) HaaXaahArgChaGlnSerSer (SEQ.ID.NO.: 6);
- g) HaaXaaSerTyrGlnSerXaa (SEQ.ID.NO.: 7);
- 20 h) HaaTyrGlnSerXaa (SEQ.ID.NO.: 8);
- i) HaaXaaSerChgGlnSerXaa (SEQ.ID.NO.: 9);
- 25 j) HaaChgGlnSerXaa (SEQ.ID.NO.: 10);

wherein Haa is a cyclic amino acid substituted with a hydrophilic moiety, Xaa is any amino acid, hArg is homoarginine, Cha is cyclohexylalanine and Chg is cyclohexylglycine.

30 In an embodiment of the instant invention, the oligopeptide comprises an oligomer that is selected from:

- a) HaaTyrGlnSerSerSerLeu (SEQ.ID.NO.: 11),

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- b) HaaXaaSerTyrGlnSerAla (SEQ.ID.NO.: 12),
- c) AlaHaaXaaSerTyrTyrSer (SEQ.ID.NO.: 13),
- 5 d) AlaAsnHaaXaaSerTyrGlnSer (SEQ.ID.NO.: 14),
- e) HaaXaaSerTyrGlnSerSerThr (SEQ.ID.NO.: 15),
- 10 f) HaaTyrGlnSerSerThr (SEQ.ID.NO.: 16),
- g) HaaXaaSerTyrGlnSerSerSer (SEQ.ID.NO.: 17),
- h) HaaTyrGlnSerSerSer (SEQ.ID.NO.: 18),
- 15 i) HaaXaaLysTyrGlnSerSerSer (SEQ.ID.NO.: 19),
- j) HaaXaaHArgTyrGlnSerSerSer (SEQ.ID.NO.: 20),
- k) HaaXaaSerTyrGlnSerSerLeu (SEQ.ID.NO.: 21);
- 20 l) HaaTyrGlnSerSerLeu (SEQ.ID.NO.: 22);
- m) HaaXaaSerTyrGlnSerLeu (SEQ.ID.NO.: 23);
- 25 n) HaaTyrGlnSerLeu (SEQ.ID.NO.: 24);
- p) HaaXaaSerTyrGlnSerNle (SEQ.ID.NO.: 25);
- q) HaaTyrGlnSerNle (SEQ.ID.NO.: 26);
- 30 r) HaaXaaSerTyrGlnSerTIC (SEQ.ID.NO.: 27);
- s) HaaTyrGlnSerTIC (SEQ.ID.NO.: 28);

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- t) HaaXaaSerChgGlnSerLeu (SEQ.ID.NO.: 29);
- u) HaaChgGlnSerLeu (SEQ.ID.NO.: 30);
- 5 v) HaaXaaSerChgGlnSerNle (SEQ.ID.NO.: 31);
- w) HaaChgGlnSerNle (SEQ.ID.NO.: 32);
- x) HaaXaaSerChgGlnSerTIC (SEQ.ID.NO.: 33);
- 10 y) HaaChgGlnSerTIC (SEQ.ID.NO.: 34);
- z) hArgChgGlnSerLeu (SEQ.ID.NO.: 35); and
- 15 aa) hArgTyrGlnSerLeu (SEQ.ID.NO.: 36).

In a more preferred embodiment of the instant invention,
the oligopeptide comprises an oligomer selected from:

- 20 a) 4-HypXaaSerTyrGlnSerSer (SEQ.ID.NO.: 37),
- b) 4-HypXaaSerTyrGlnSerAla (SEQ.ID.NO.: 38),
- c) Ala-4-HypXaaSerTyrTyrSer (SEQ.ID.NO.: 39),
- 25 d) AlaAsn4-HypXaaSerTyrGlnSer (SEQ.ID.NO.: 40),
- e) 4-HypXaaSerTyrGlnSerSerThr (SEQ.ID.NO.: 41),
- 30 f) 4-HypTyrGlnSerSerThr (SEQ.ID.NO.: 42),
- g) 4-HypXaaSerTyrGlnSerSerSer (SEQ.ID.NO.: 43),
- h) 4-HypTyrGlnSerSerSer (SEQ.ID.NO.: 44),

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- i) 4-HypXaaLysTyrGlnSerSerSer (SEQ.ID.NO.: 45),
j) 4-HypXaaHArgTyrGlnSerSerSer (SEQ.ID.NO.: 46),
5 k) 4-HypXaaSerTyrGlnSerSerLeu (SEQ.ID.NO.: 47);
l) 4-HypTyrGlnSerSerLeu (SEQ.ID.NO.: 48);
10 m) 4-HypXaaSerTyrGlnSerLeu (SEQ.ID.NO.: 49);
n) 4-HypTyrGlnSerLeu (SEQ.ID.NO.: 50);
p) 4-HypXaaSerTyrGlnSerNle (SEQ.ID.NO.: 51);
15 q) 4-HypTyrGlnSerNle (SEQ.ID.NO.: 52);
r) 4-HypXaaSerTyrGlnSerTIC (SEQ.ID.NO.: 53);
20 s) 4-HypTyrGlnSerTIC (SEQ.ID.NO.: 54);
t) 4-HypXaaSerChgGlnSerLeu (SEQ.ID.NO.: 55);
u) 4-HypChgGlnSerLeu (SEQ.ID.NO.: 56);
25 v) 4-HypXaaSerChgGlnSerNle (SEQ.ID.NO.: 57);
w) 4-HypChgGlnSerNle (SEQ.ID.NO.: 58);
30 x) 4-HypXaaSerChgGlnSerTIC (SEQ.ID.NO.: 59);
y) 4-HypChgGlnSerTIC (SEQ.ID.NO.: 60);

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wherein 4-Hyp is 4-hydroxyproline, Xaa is any amino acid, hArg is homoarginine, Cha is cyclohexylalanine and Chg is cyclohexylglycine.

Preferably Xaa in the more preferred embodiment is selected from Ala, Ser and Ile.

- 5 The phrase "oligomers that comprise an amino acid sequence" as used hereinabove, and elsewhere in the Detailed Description of the Invention, describes oligomers of from about 3 to about 100 amino acids residues which include in their amino acid sequence the specific amino acid sequence described and which
10 are therefore proteolytically cleaved within the amino acid sequence described by free PSA. Preferably, the oligomer is from 5 to 10 amino acid residues. Thus, for example, the following oligomer:
hArgSer4-HypChgGlnSerLeu (SEQ.ID.NO.: 61);
comprises the amino acid sequence:
15 4-HypChgGlnSerLeu (SEQ.ID.NO.: 56);
and would therefore come within the instant invention.

- A person of ordinary skill in the peptide chemistry art would readily appreciate that certain amino acids in a biologically active oligopeptide may be replaced by other homologous, isosteric and/or
20 isoelectronic amino acids wherein the biological activity of the original oligopeptide has been conserved in the modified oligopeptide. Certain unnatural and modified natural amino acids may also be utilized to replace the corresponding natural amino acid in the oligopeptides of the instant invention. Thus, for example, tyrosine may be replaced by
25 3-iodotyrosine, 2-methyltyrosine, 3-fluorotyrosine, 3-methyltyrosine and the like. Further for example, lysine may be replaced with N'-(2-imidazolyl)lysine and the like. The following list of amino acid replacements is meant to be illustrative and is not limiting:

<u>Original Amino Acid</u>	<u>Replacement Amino Acid(s)</u>
Ala	Gly
Arg	Lys, Ornithine
Asn	Gln
Asp	Glu

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Glu	Asp
Gln	Asn
Gly	Ala
Ile	Val, Leu, Met, Nle
Leu	Ile, Val, Met, Nle
Lys	Arg, Ornithine
Met	Leu, Ile, Nle, Val
Ornithine	Lys, Arg
Phe	Tyr, Trp
Ser	Thr
Thr	Ser
Trp	Phe, Tyr
Tyr	Phe, Trp
Val	Leu, Ile, Met, Nle

Thus, for example, the following oligopeptides may be synthesized by techniques well known to persons of ordinary skill in the art and would be expected to be proteolytically cleaved by free PSA when incorporated in a conjugate of this invention:

- Asn4-HypIleSerTyrGlnIser (SEQ.ID.NO.: 62)
 Asn4-HypValSerTyrGlnIser (SEQ.ID.NO.: 63)
 4-HypAlaSerTyrGlnIserSer (SEQ.ID.NO.: 64)
 10 (3,4-dihydroxyproline)AlaSerTyrGln IserSer (SEQ.ID.NO.: 65)
 3-hydroxyprolineSerChgGlnIser (SEQ.ID.NO.: 66)
 4-HypAlaSerChgGlnIserSer (SEQ.ID.NO.: 67).

The inclusion of the symbol "I" within an amino acid sequence indicates the point within that sequence where the oligopeptide is proteolytically cleaved by free PSA.

The compounds of the present invention may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. Unless otherwise

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specified, named amino acids are understood to have the natural "L" stereoconfiguration

The following abbreviations are utilized in the specification and tables to denote the indicated amino acids and moieties:

5	hR or hArg:	homoarginine
	hY or hTyr:	homotyrosine
	Cha:	cyclohexylalanine
	Amf:	4-aminomethylphenylalanine
10	DPL:	2-(4,6-dimethylpyrimidinyl)lysine
	(imidazolyl)K:	N'-(2-imidazolyl)lysine
	Me ₂ PO ₃ -Y:	O-dimethylphosphotyrosine
	O-Me-Y:	O-methyltyrosine
	TIC:	1,2,3,4-tetrahydro-3-isoquinoline
15		carboxylic acid
	DAP:	1,3-diaminopropane
	TFA:	trifluoroacetic acid
	AA:	acetic acid
	3PAL	3-pyridyl-alanine
20	4-Hyp	4-hydroxyproline
	Abu	alpha-aminobutyric acid
	Thi	thienylalanine

It is well known in the art, and understood in the instant invention, that peptidyl therapeutic agents such as the instant oligopeptide-cytotoxic agent conjugates preferably have the terminal amino moiety of any oligopeptide substituent protected with a suitable protecting group, such as acetyl, benzoyl, pivaloyl and the like. Such protection of the terminal amino group reduces or eliminates the enzymatic degradation of such peptidyl therapeutic agents by the action of amino peptidases which are present in the blood plasma of warm blooded animals.

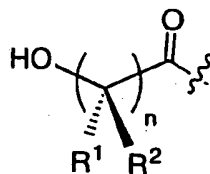
Such protecting groups also include a hydrophilic

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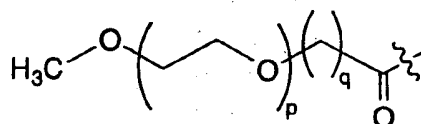
blocking groups, which are chosen based upon the presence of hydrophilic functionality. Blocking groups that increase the hydrophilicity of the conjugates and therefore increase the aqueous solubility of the conjugates include but are not limited to hydroxylated alkanoyl, polyhydroxylated alkanoyl, hydroxylated aroyl, polyhydroxylated aroyl, polyethylene glycol, glycosylates, sugars and crown ethers. N-Terminus unnatural amino acid moieties may also ameliorate such enzymatic degradation by amino peptidases.

Preferably the N-terminus protecting group is selected from

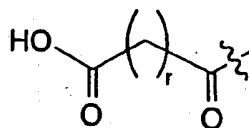
- a) acetyl;
b)



15 c)



d)



20

wherein:

R¹ and R² are independently selected from:

- a) hydrogen,
b) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halogen, C₁-C₆ perfluoroalkyl, R³O-,

25

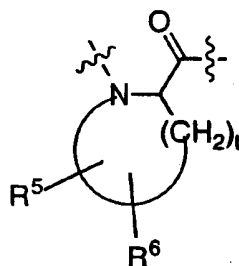
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- $R^3C(O)NR^3-$, $(R^3)_2NC(O)-$, $R^3_2N-C(NR^3)-$, $R^4S(O)_mNH$,
 CN , NO_2 , $R^3C(O)-$, N_3 , $-N(R^3)_2$, or $R^4OC(O)NR^3-$,
 c) unsubstituted C1-C6 alkyl,
 d) substituted C1-C6 alkyl wherein the substituent on the
 substituted C1-C6 alkyl is selected from unsubstituted or
 substituted aryl, unsubstituted or substituted heterocyclic,
 C3-C10 cycloalkyl, C2-C6 alkenyl, C2-C6 alkynyl, R^3O- ,
 $R^4S(O)_mNH$, $R^3C(O)NR^3-$, $(R^3)_2NC(O)-$, $R^3_2N-C(NR^3)-$,
 CN , $R^3C(O)-$, N_3 , $-N(R^3)_2$, and $R^4OC(O)-NR^3-$; or
 R1 and R2 are combined to form $-(CH_2)_s-$ wherein one of the
 carbon atoms is optionally replaced by a moiety selected
 from: O, $S(O)_m$, $-NC(O)-$, NH and $-N(COR^4)-$;
 R³ is selected from: hydrogen, aryl, substituted aryl, heterocycle,
 substituted heterocycle, C1-C6 alkyl and C3-C10
 cycloalkyl;
 R⁴ is selected from: aryl, substituted aryl, heterocycle, substituted
 heterocycle, C1-C6 alkyl and C3-C10 cycloalkyl;
 m is 0, 1 or 2;
 n is 1, 2, 3 or 4;
 p is zero or an integer between 1 and 100;
 q is 0 or 1, provided that if p is zero, q is 1;
 r is an integer between 1 and 10; and
 s is 3, 4 or 5.

Preferably, r is 1, 2 or 3.

The oligopeptides of the instant conjugates comprise a cyclic amino acid substituted with a hydrophilic moiety, previously represented by the term "Haa", which may also be represented by the formula:

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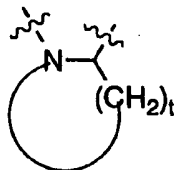
wherein:

R5 is selected from HO- and C1-C6 alkoxy;

5 R6 is selected from hydrogen, halogen, C1-C6 alkyl, HO- and C1-C6 alkoxy; and

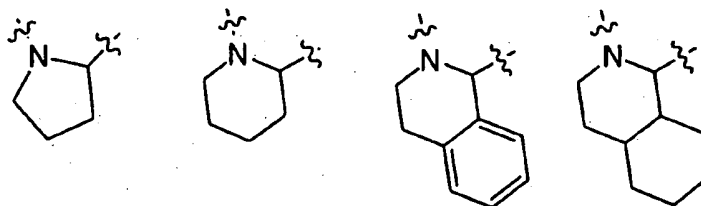
t is 3 or 4.

10 The structure



represents a cyclic amine moiety having 5 or 6 members in the ring, such a cyclic amine which may be optionally fused to a phenyl or cyclohexyl ring. Examples of such a cyclic amine moiety include,

15 but are not limited to, the following specific structures:



The conjugates of the present invention may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical

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isomers, being included in the present invention. When any variable (e.g. aryl, heterocycle, R^3 etc.) occurs more than one time in any constituent, its definition on each occurrence is independent of every other occurrence. For example, $HO(CR^1R^2)_2-$ represents $HOCH_2CH_2-$,
5 $HOCH_2CH(OH)-$, $HOCH(CH_3)CH(OH)-$, etc. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" and the alkyl portion of aralkyl and similar terms, is intended to include both branched and straight-chain
10 saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge.

As used herein, "cycloalkyl" is intended to include non-aromatic cyclic hydrocarbon groups having the specified number of
15 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

"Alkenyl" groups include those groups having the specified number of carbon atoms and having one or several double bonds. Examples of alkenyl groups include vinyl, allyl, isopropenyl, pentenyl,
20 hexenyl, heptenyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, isoprenyl, farnesyl, geranyl, geranylgeranyl and the like.

"Alkynyl" groups include those groups having the specified number of carbon atoms and having one triple bonds. Examples of
25 alkynyl groups include acetylene, 2-butyne, 2-pentyne, 3-pentyne and the like.

"Halogen" or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "aryl," and the aryl portion of aralkyl and
30 aroyl, is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl.

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The term heterocycle or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdiny, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl.

As used herein in the terms "substituted C₁₋₈ alkyl", "substituted aryl" and "substituted heterocycle" include moieties containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound. Such additional substituents are selected from F, Cl, Br, CF₃, NH₂, N(C₁-C₆ alkyl)₂, NO₂, CN, (C₁-C₆ alkyl)O-, -OH, (C₁-C₆ alkyl)S(O)_m-, (C₁-C₆ alkyl)C(O)NH-, H₂N-C(NH)-, (C₁-C₆ alkyl)C(O)-, (C₁-C₆ alkyl)OC(O)-, N₃, (C₁-C₆ alkyl)OC(O)NH- and C₁-C₂₀ alkyl.

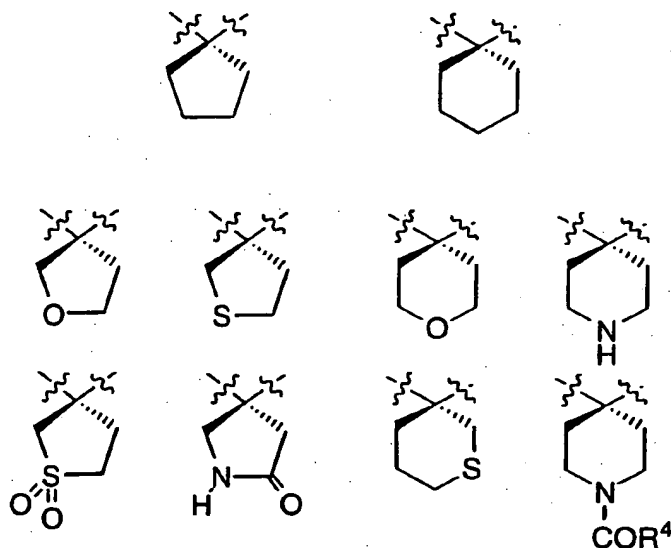
The term "an integer between 1 and 10" represents the numbers 1 and 10 as well as those integers between those numbers. The term "an integer between 1 and 100" represents the numbers 1

- 18 -

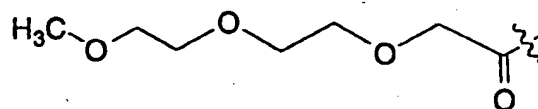
and 100 as well as those integers between those numbers.

When R^1 and R^2 are combined to form $-(CH_2)_s-$, the cyclic moieties and heteroatom-containing cyclic moieties so defined include, but are not limited to:

5

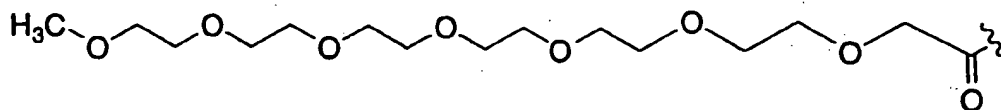


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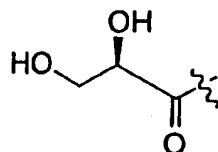
and the term PEG(6) represents

15



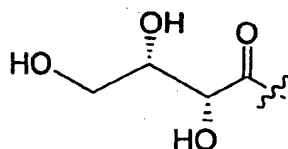
As used herein, the term "(2R)(2,3-dihydroxypropionyl)" represents the following structure:

- 19 -



As used herein, the term "(2R,3S) 2,3,4-trihydroxybutanoyl" represents the following structure:

5



Because the conjugates of the invention can be used for modifying a given biological response, cytotoxic agent is not to be construed as limited to classical chemical therapeutic agents. For example, the cytotoxic agent may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

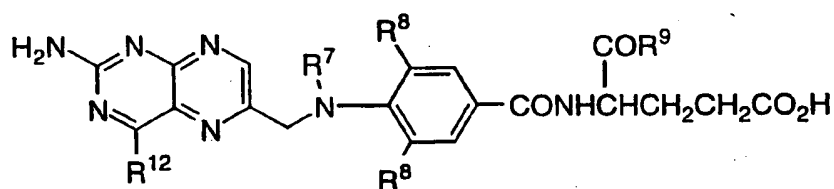
The preferred cytotoxic agents include, in general, alkylating agents, antiproliferative agents, tubulin binding agents and the like. Preferred classes of cytotoxic agents include, for example, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the pteridine family of drugs, diynes and the podophyllotoxins. Particularly useful members of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C, porfiromycin, 5-fluorouracil,

- 20 -

- 6-mercaptopurine, cytosine arabinoside, podophyllotoxin, or podophyllotoxin derivatives such as etoposide or etoposide phosphate, melphalan, vinblastine, vincristine, leurosine, vindesine, leurosine, taxol and the like. Other useful cytotoxic agents include estramustine, cisplatin and cyclophosphamide. One skilled in the art may make chemical modifications to the desired cytotoxic agent in order to make reactions of that compound more convenient for purposes of preparing conjugates of the invention.

- A highly preferred group of cytotoxic agents for the present invention include drugs of the following formulae:

THE METHOTREXATE GROUP OF FORMULA(1):



(1)

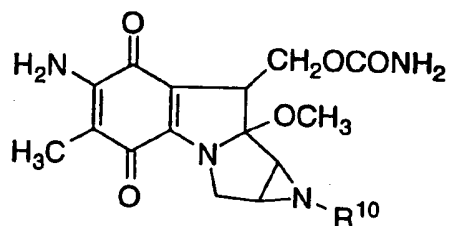
15

in which

- R¹² is amino or hydroxy;
 R⁷ is hydrogen or methyl;
 R⁸ is hydrogen, fluoro, chloro, bromo or iodo;
 R⁹ is hydroxy or a moiety which completes a salt of the carboxylic acid;

20

THE MITOMYCIN GROUP OF FORMULA (2):



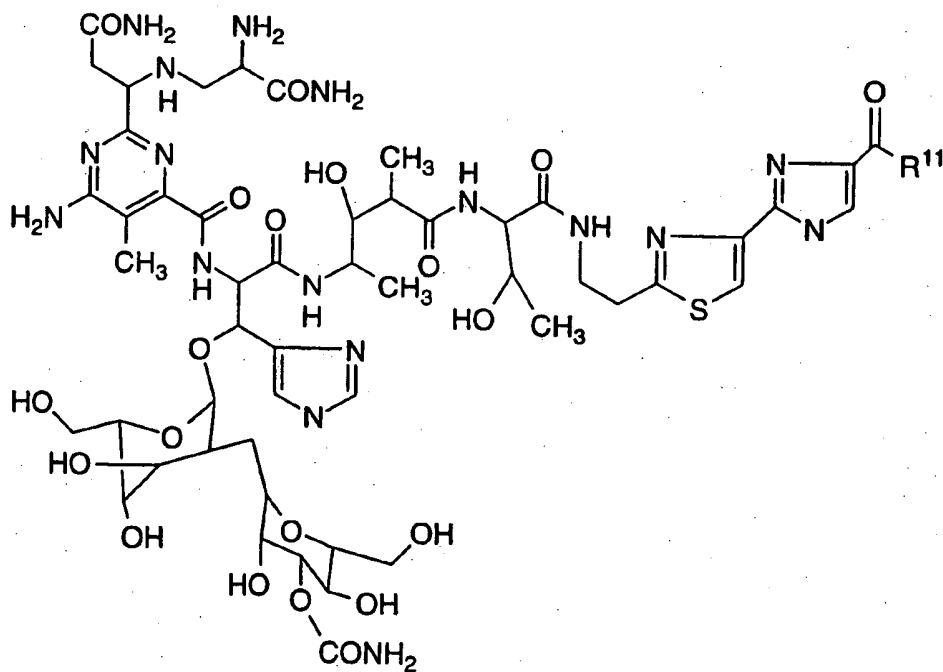
(2)

in which

R¹⁰ is hydrogen or methyl;

5

THE BLEOMYCIN GROUP OF FORMULA (3)

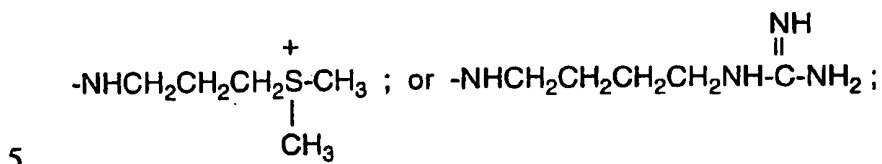


(3)

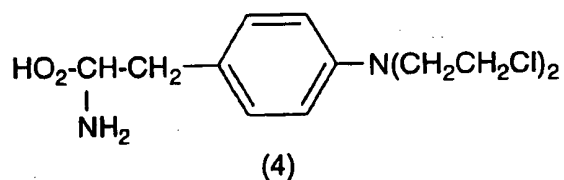
- 22 -

in which

R¹¹ is hydroxy, amino, C₁-C₃ alkylamino, di(C₁-C₃ alkyl)amino, C₄-C₆ polymethylene amino,

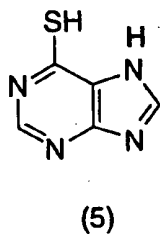


MELPHALAN OF FORMULA (4):

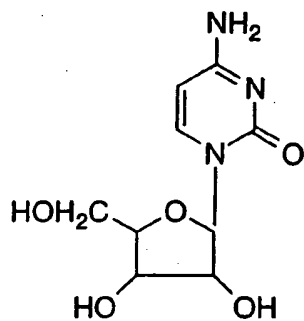


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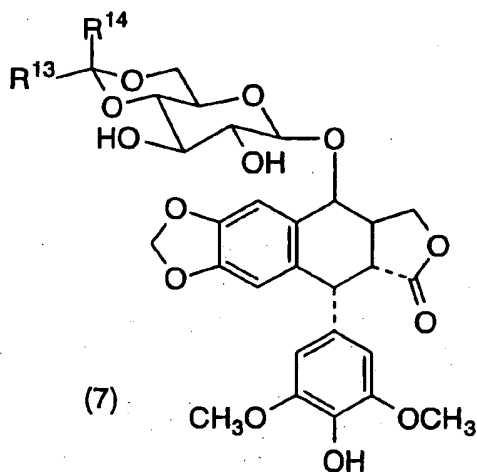
6-MERCAPTOPURINE OF FORMULA (5):



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A CYTOSINE ARABINOSIDE OF FORMULA (6):

(6)

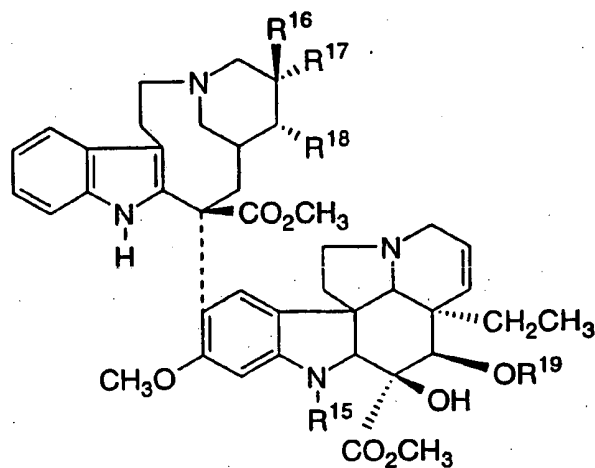
5 THE PODOPHYLLOTOXINS OF FORMULA(7):

(7)

in which

- 10 R¹³ is hydrogen or methyl;
 R¹⁴ is methyl or thienyl;
 or a phosphate salt thereof;

- 24 -

THE VINCA ALKALOID GROUP OF DRUGS OF FORMULA (8):

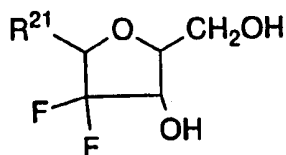
(8)

5

in which

- R¹⁵ is H, CH₃ or CHO; when R¹⁷ and R¹⁸ are taken singly;
 R¹⁸ is H, and one of R¹⁶ and R¹⁷ is ethyl and the other is H
 or OH; when R¹⁷ and R¹⁸ are taken together with the
 carbons to which they are attached, they form an
 oxirane ring in which case R¹⁶ is ethyl;
 R¹⁹ is hydrogen, (C₁-C₃ alkyl)-CO, or chlorosubstituted
 (C₁-C₃ alkyl)-CO;

10

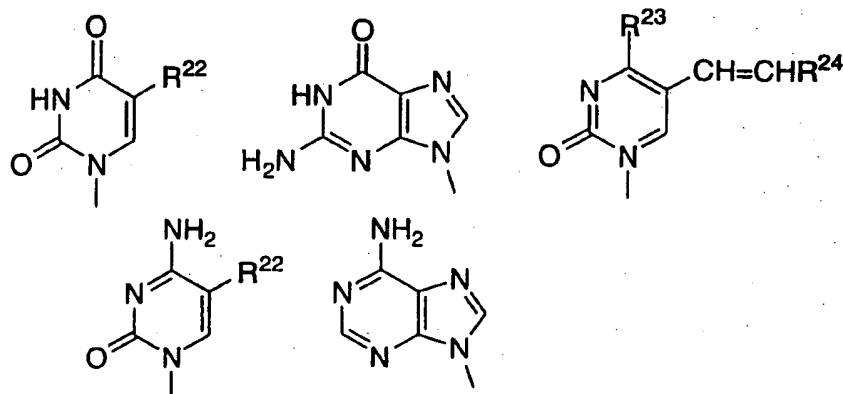
15 DIFLUORONUCLEOSIDES OF FORMULA (9):

(9)

in which

- 25 -

R²¹ is a base of one of the formulae:



in which

R²² is hydrogen, methyl, bromo, fluoro, chloro or iodo;

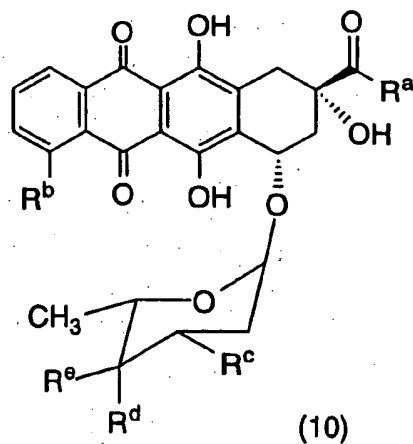
5 R²³ is -OH or -NH₂;

R²⁴ is hydrogen, bromo, chloro or iodo;

or,

THE ANTHRACYCLINES ANTIBIOTICS OF FORMULA (10):

10

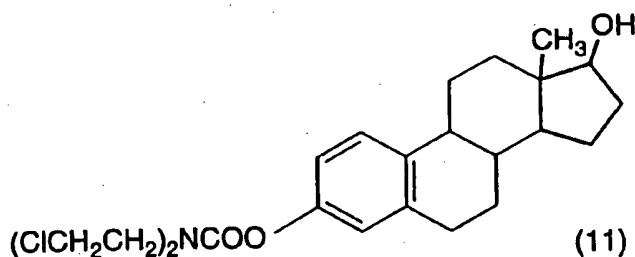
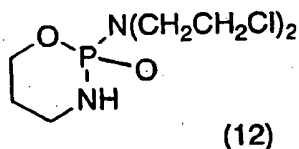


wherein

15 R^a is -CH₃, -CH₂OH, -CH₂OCO(CH₂)₃CH₃, or
-CH₂OCOCH(OC₂H₅)₂;

- 26 -

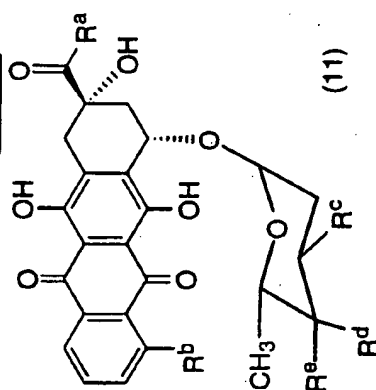
- R^b is $-\text{OCH}_3$, $-\text{OH}$ or $-\text{H}$;
 R^c is $-\text{NH}_2$, $-\text{NHCOCF}_3$, 4-morpholinyl, 3-cyano-4-morpholinyl, 1-piperidinyl, 4-methoxy-1-piperidinyl, benzylamine, dibenzylamine, cyanomethylamine, or 1-cyano-2-methoxyethyl amine;
 R^d is $-\text{OH}$ $-\text{OTHP}$ or $-\text{H}$; and
 R^e is $-\text{OH}$ or $-\text{H}$ provided that R^6 is not $-\text{OH}$ when R^5 is $-\text{OH}$ or $-\text{OTHP}$.

10 ESTRAMUSTINE (11)15 CYCLOPHOSPHAMIDE (12)

The most highly preferred drugs are the anthracycline
 antibiotic agents of Formula (10), described previously. One skilled
 in the art understands that this structural formula includes compounds
 which are drugs, or are derivatives of drugs, which have acquired in
 the art different generic or trivial names. Table 1, which follows,
 represents a number of anthracycline drugs and their generic or trivial
 names and which are especially preferred for use in the present
 invention.

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Table 1



Compound	R^a	R^b	R^c	R^d	R^e
daunorubicin ^a	CH ₃	OCH ₃	NH ₂	OH	H
doxorubicin ^b	CH ₂ OH	OCH ₃	NH ₂	OH	H
detorubicin	CH ₂ OCOCH(OC ₂ H ₅) ₂	OCH ₃	NH ₂	OH	H
carminomycin	CH ₃	OH	NH ₂	OH	H
idarubicin	CH ₃	H	NH ₂	OH	H
epirubicin	CH ₂ OH	OCH ₃	NH ₂	OH	OH
esorubicin	CH ₂ OH	OCH ₃	NH ₂	H	H
THP	CH ₂ OH	OCH ₃	NH ₂	OTHP	H
AD-32	CH ₂ OCO(CH ₂) ₃ CH ₃	OCH ₃	NHCOCF ₃	OH	H

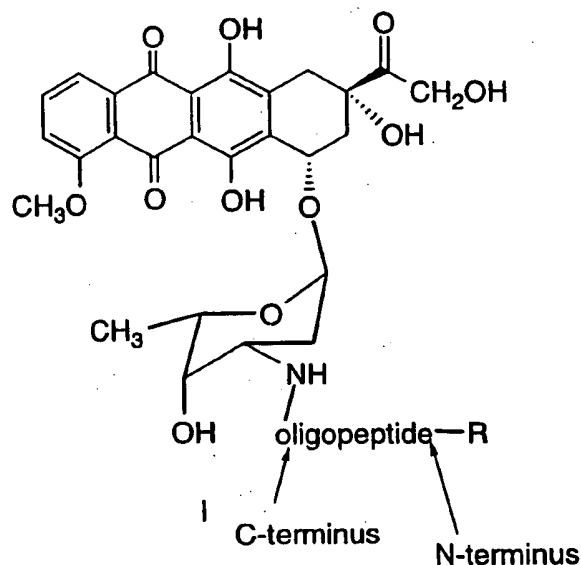
^a"daunomycin" is an alternative name for daunorubicin

^b"adriamycin" is an alternative name for doxorubicin

- 28 -

Of the compounds shown in Table 1, the most highly preferred cytotoxic agents are doxorubicin, vinblastine and desacetylvinblastine. Doxorubicin (also referred to herein as "DOX") is that anthracycline of Formula (10) in which R^a is -CH₂OH, R^b is -OCH₃, R^c is -NH₂, R^d is -OH, and R^e is -H.

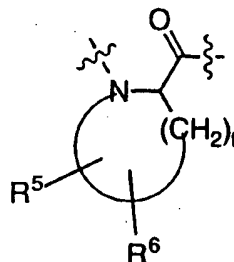
The oligopeptide-cytotoxic agent conjugate of the instant invention wherein the cytotoxic agent is the preferred cytotoxic agent doxorubicin may be described by the general formula I below:



wherein:

oligopeptide is an oligopeptide which is selectively recognized by the free prostate specific antigen (PSA) and is capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen, wherein the oligopeptide comprises a cyclic amino acid of the formula:

- 29 -



and wherein

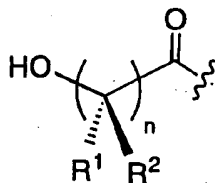
the C-terminus carbonyl is covalently bound to the amine of doxorubicin;

5

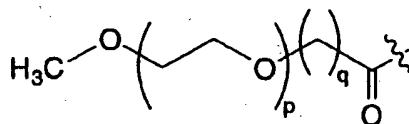
R is selected from

- a) hydrogen,
b) $-(C=O)R^{1a}$,
c)

10

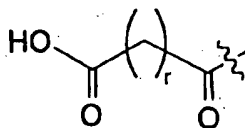


d)



15

e)



R^1 and R^2 are independently selected from: hydrogen, OH, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ aralkyl and aryl;

20

- 30 -

R^{1a} is C₁-C₆-alkyl, hydroxylated aryl, polyhydroxylated aryl or aryl,

R⁵ is selected from HO- and C₁-C₆ alkoxy;

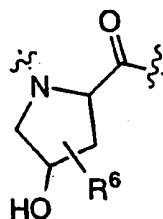
- 5 R⁶ is selected from hydrogen, halogen, C₁-C₆ alkyl, HO- and C₁-C₆ alkoxy; and

- n is 1, 2, 3 or 4;
 p is zero or an integer between 1 and 100;
 10 q is 0 or 1, provided that if p is zero, q is 1;
 r is an integer between 1 and 10; and
 t is 3 or 4;

or a pharmaceutically acceptable salt thereof.

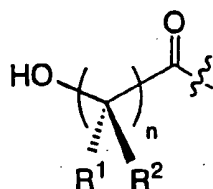
- 15 In a preferred embodiment of the oligopeptide-cytotoxic agent conjugate:

the cyclic amino acid is



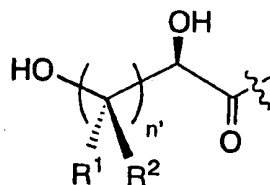
- 20 R is selected from

- a) hydrogen,
 b) $-(C=O)R^{1a}$,
 c)

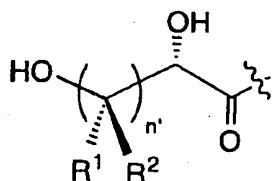


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d)

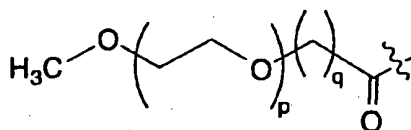


e)



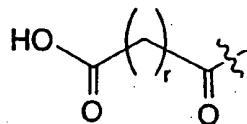
5

f)



10

g)



R^1 and R^2 are independently selected from: hydrogen, C₁-C₆ alkyl and aryl;

15

R^{1a} is C₁-C₆-alkyl or aryl,

n is 1, 2, 3 or 4;

n' is 0, 1, 2 or 3;

20 p is zero or an integer between 1 and 14;

q is 0 or 1, provided that if p is zero, q is 1;

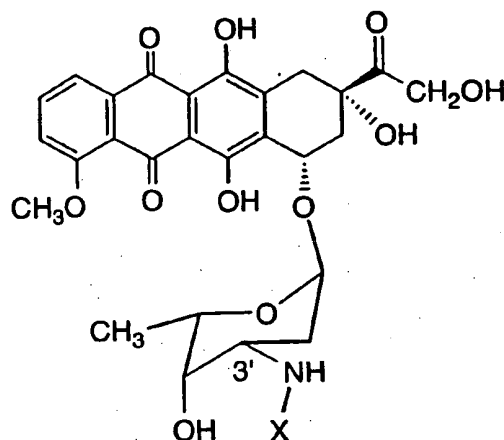
r is an integer between 1 and 10; and

t is 3;

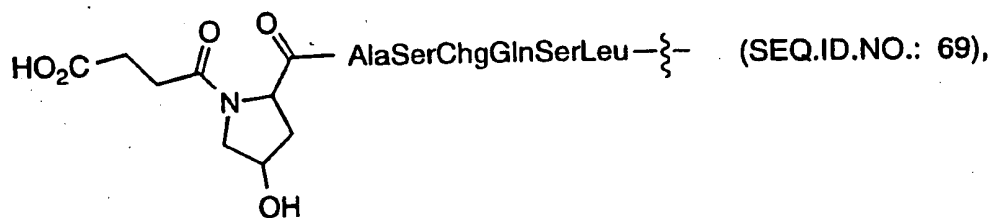
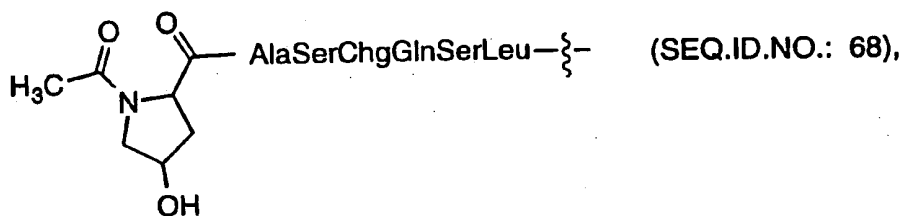
- 32 -

or an optical isomer or a pharmaceutically acceptable salt thereof.

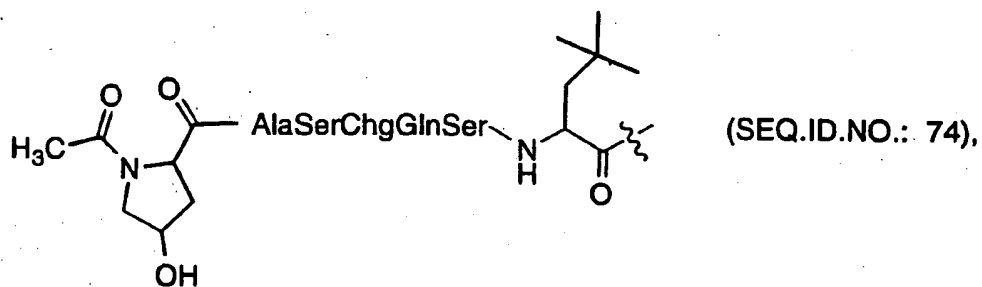
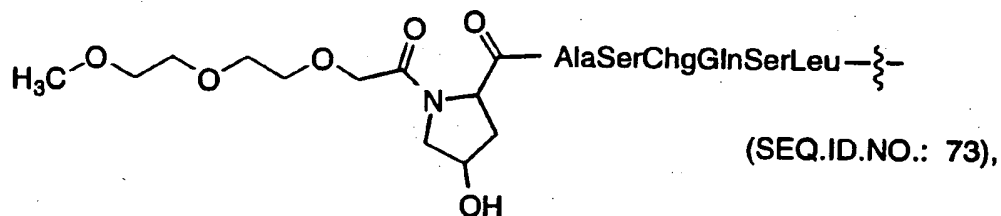
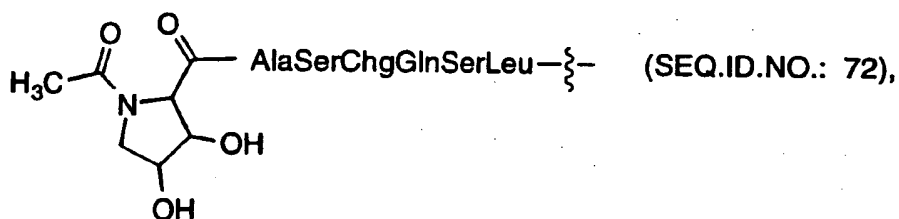
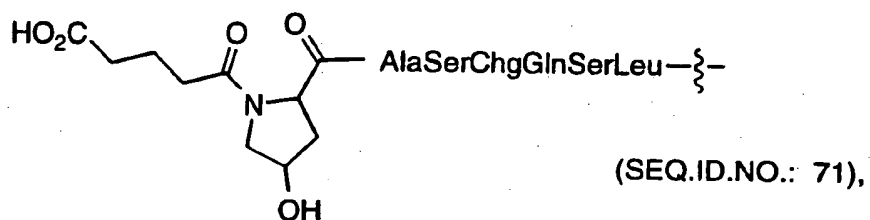
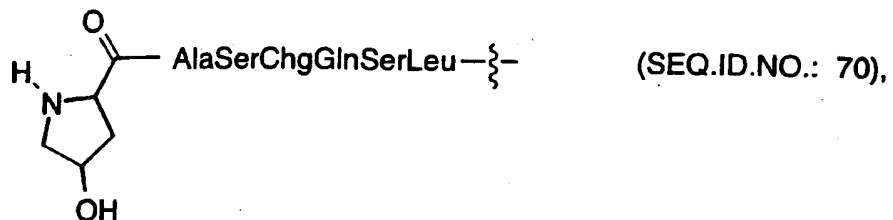
The following compounds are specific examples of the oligopeptide-cytotoxic agent conjugate of the instant invention:



wherein X is:



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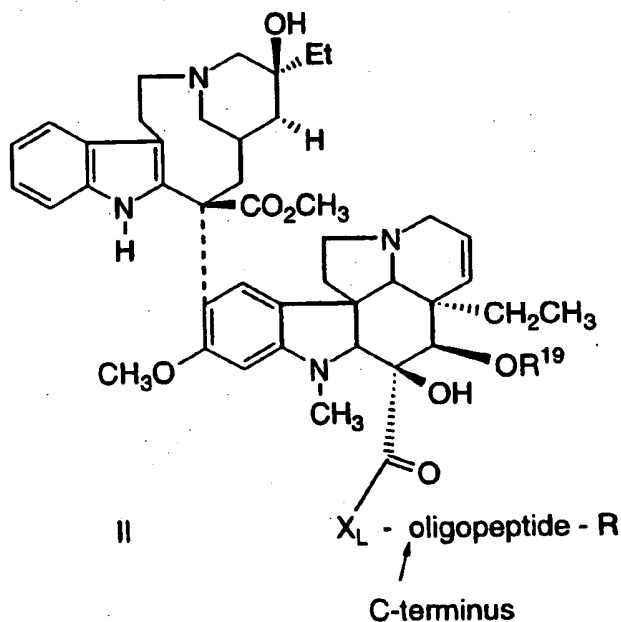
	SEQ. ID. NO.
Succinyl-(4-Hyp)ASChgQ-SV-DOX (3')	75
Glutaryl-(4-Hyp)ASChgQ-SV-DOX (3')	76
Glutaryl-(4-Hyp)ASChgQ-SI-DOX (3')	77
Succinyl-(4-Hyp)SSChgQ-SI-DOX (3')	78

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Succinyl-(4-Hyp)ASChgQ-SI-DOX (3')	79
Succinyl-(4-Hyp)ASChgQ-SABu-DOX (3')	80
Glutaryl-(4-Hyp)SSChgQ-SI-DOX (3')	81
Glutaryl-(4-Hyp)SSChgQ-SL-DOX (3')	82
PEG(2)-(4-Hyp)SSChgQ-SL-DOX (3')	83
Succinyl-(4-Hyp)ASChgQ-SThi-DOX (3')	84
PEG(4)-(4-Hyp)-SSChgQ-SL-DOX (3')	85
PEG(2)-(4-Hyp)ASChgQ-SThi-DOX(3')	86
Succinyl-3,4-(diOH)PASChgQ-SL-DOX (3')	87
Malonyl-(4-Hyp)ASChgQ-SL-DOX (3')	88

or an optical isomer or pharmaceutically acceptable salt thereof.

- The oligopeptide-cytotoxic agent conjugate of the instant invention wherein the cytotoxic agent is the preferred cytotoxic agent vinblastine or desacetylvinblastine may be described by the general formula II below:

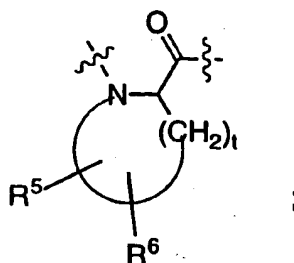


- 10 wherein:

oligopeptide is an oligopeptide which is specifically recognized by the free prostate specific antigen (PSA) and is capable of being proteolytic-

- 35 -

ally cleaved by the enzymatic activity of the free prostate specific antigen, and the oligopeptide comprises a cyclic amino acid of the formula:

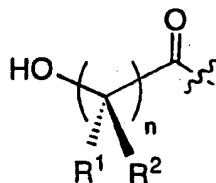


5 XL is - NH - (CH₂)_u - NH -

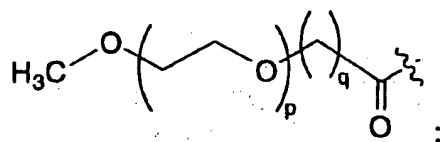
R is selected from

- a) hydrogen,
b) -(C=O)R^{1a},
c)

10

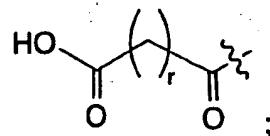


d)



15

e)



R¹ and R² are independently selected from: hydrogen, OH, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ aralkyl and aryl;

20

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R^{1a} is C₁-C₆-alkyl, hydroxylated aryl, polyhydroxylated aryl or aryl,

R¹⁹ is hydrogen, (C1-C3 alkyl)-CO, or chlorosubstituted (C1-C3 alkyl)-CO;

5

n is 1, 2, 3 or 4;

p is zero or an integer between 1 and 100;

q is 0 or 1, provided that if **p** is zero, **q** is 1;

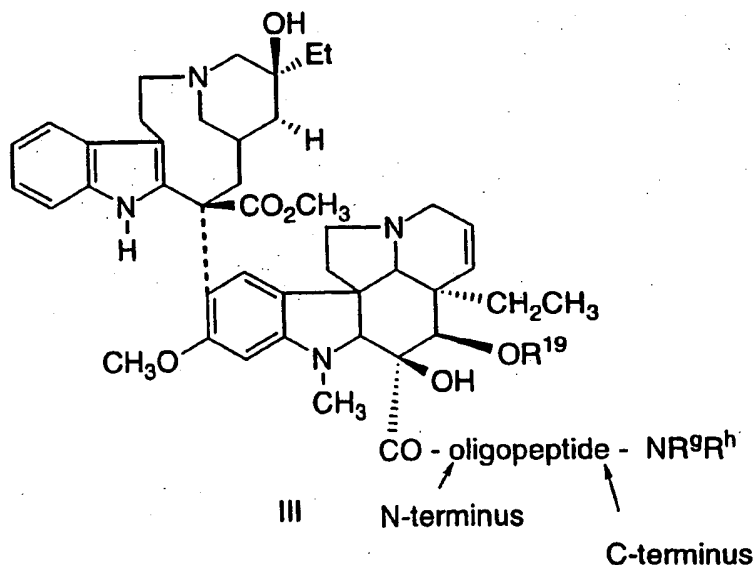
r is 1, 2 or 3;

10 t is 3 or 4;

u is 1, 2, 3, 4 or 5,

or the pharmaceutically acceptable salt thereof.

Another embodiment of the oligopeptide-cytotoxic agent conjugate of the instant invention wherein the cytotoxic agent is the preferred cytotoxic agent vinblastine or desacetylvinblastine may be described by the general formula III below:



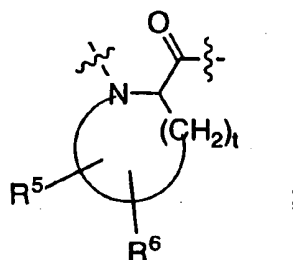
wherein:

20

oligopeptide is an oligopeptide which is specifically recognized

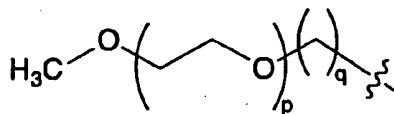
- 37 -

by the free prostate specific antigen (PSA) and is capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen, and the oligopeptide comprises a cyclic amino acid of the formula:



5

R^g and R^h are independently selected from: hydrogen, C1-C6-alkyl, -C1-C6-alkyl-OH, -C1-C6-alkyl-di-OH, -C1-C6-alkyl-tri-OH and



10

provided that at least one R^d and R^e are not hydrogen or C1-C6-alkyl, or

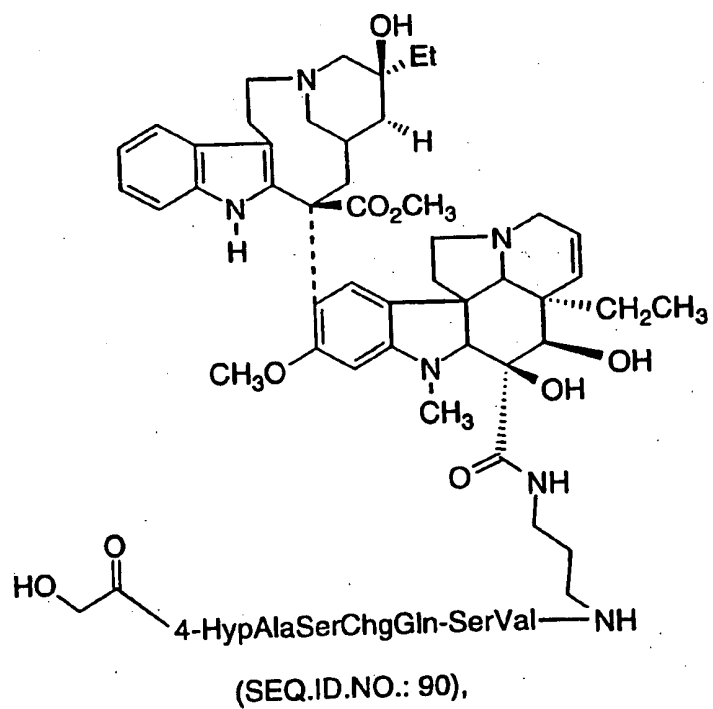
15 R^g and R^h are combined to form a -CH₂CH₂OCH₂CH₂- diradical;

R^{19} is hydrogen, (C1-C3 alkyl)-CO, or chlorosubstituted (C1-C3 alkyl)-CO;

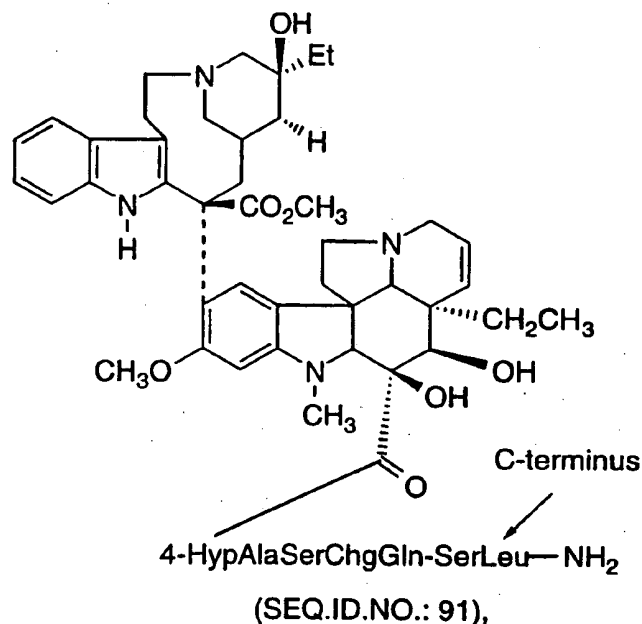
20 p is zero or an integer between 1 and 100;
 q is 0 or 1, provided that if p is zero, q is 1;

or the pharmaceutically acceptable salt thereof.

25 The following compounds are specific examples of the oligopeptide-desacetylvinblastine conjugate of the instant invention:



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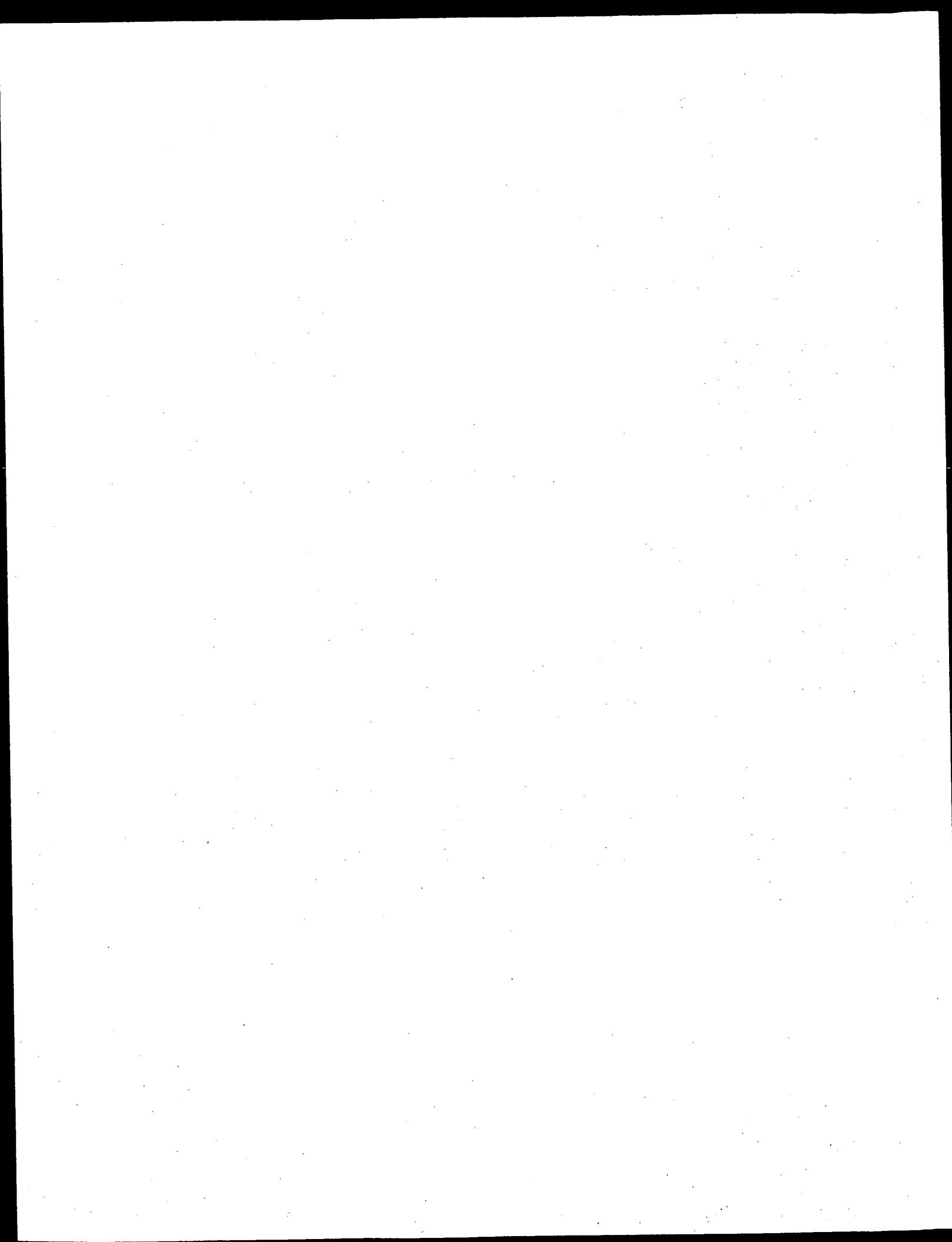


or an optical isomer or pharmaceutically acceptable salt thereof.

The oligopeptides, peptide subunits and peptide derivatives (also termed "peptides") of the present invention can be synthesized from their constituent amino acids by conventional peptide synthesis techniques, preferably by solid-phase technology. The peptides are then purified by reverse-phase high performance liquid chromatography (HPLC).

Standard methods of peptide synthesis are disclosed, for example, in the following works: Schroeder *et al.*, "The Peptides", Vol. I, Academic Press 1965; Bodansky *et al.*, "Peptide Synthesis", Interscience Publishers, 1966; McOmie (ed.) "Protective Groups in Organic Chemistry", Plenum Press, 1973; Barany *et al.*, "The Peptides: Analysis, Synthesis, Biology" 2, Chapter 1, Academic Press, 1980, and Stewart *et al.*, "Solid Phase Peptide Synthesis", Second Edition, Pierce Chemical Company, 1984. The teachings of these works are hereby incorporated by reference.

The suitably substituted cyclic amino acid having a hydrophilic substituent, which may be incorporated into the instant conjugates by standard peptide synthesis techniques, is itself either



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commercially available or is readily synthesized by techniques well known in the art or described herein. Thus syntheses of suitably substituted prolines are described in the following articles and references cited therein: J. Ezquerro et al., *J. Org. Chem.* 60:2925-2930 (1995); P. Gill and W. D. Lubell, *J. Org. Chem.*, 60:2658-2659 (1995); and M. W. Holladay et al., *J. Med. Chem.*, 34:457-461 (1991). The teachings of these works are hereby incorporated by reference.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The conjugates of the instant invention which comprise the oligopeptide containing the PSA cleavage site and a cytotoxic agent may similarly be synthesized by techniques well known in the medicinal chemistry art. For example, a free amine moiety on the cytotoxic agent may be covalently attached to the oligopeptide at the carboxyl terminus such that an amide bond is formed. Similarly, an amide bond may be formed by covalently coupling an amine moiety of the oligopeptide and a carboxyl moiety of the cytotoxic agent. For these purposes a reagent such as 2-(1H-benzotriazol-1-yl)-1,3,3-tetramethyluronium hexafluorophosphate (known as HBTU) and 1-hydroxybenzotriazole hydrate (known as HOBT), dicyclohexylcarbodiimide (DCC), N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide (EDC), diphenylphosphoryl azide (DPPA), benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium

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hexafluorophosphate (BOP) and the like, used in combination or singularly, may be utilized.

Furthermore, the instant conjugate may be formed by a non-peptidyl bond between the PSA cleavage site and a cytotoxic agent.

- 5 For example, the cytotoxic agent may be covalently attached to the carboxyl terminus of the oligopeptide via a hydroxyl moiety on the cytotoxic agent, thereby forming an ester linkage. For this purpose a reagent such as a combination of HBTU and HOBT, a combination of BOP and imidazole, a combination of DCC and DMAP, and the like
10 may be utilized. The carboxylic acid may also be activated by forming the nitrophenyl ester or the like and reacted in the presence of DBU (1,8-diazabicyclo[5,4,0]undec-7-ene).

- The instant conjugate may also be formed by attachment of the oligopeptide to the cytotoxic agent via a linker unit. Such linker
15 units include, for example, a biscarbonyl alkyl diradical whereby an amine moiety on the cytotoxic agent is connected with the linker unit to form an amide bond and the amino terminus of the oligopeptide is connected with the other end of the linker unit also forming an amide bond. Conversely, a diaminoalkyl diradical linker unit, whereby a
20 carbonyl moiety on the cytotoxic agent is covalently attached to one of the amines of the linker unit while the other amine of the linker unit is covalently attached to the C terminus of the oligopeptide, may also be useful. Other such linker units which are stable to the physiological environment when not in the presence of free PSA, but are cleavable
25 upon the cleavage of the PSA proteolytic cleavage site, are also envisioned. Furthermore, linker units may be utilized that, upon cleavage of the PSA proteolytic cleavage site, remain attached to the cytotoxic agent but do not significantly decrease the cytotoxic activity of such a post-cleavage cytotoxic agent derivative when compared with
30 an unmodified cytotoxic agent.

One skilled in the art understands that in the synthesis of compounds of the invention, one may need to protect various reactive functionalities on the starting compounds and intermediates while a desired reaction is carried out on other portions of the molecule. After

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the desired reactions are complete, or at any desired time, normally such protecting groups will be removed by, for example, hydrolytic or hydrogenolytic means. Such protection and deprotection steps are conventional in organic chemistry. One skilled in the art is referred to

5 Protective Groups in Organic Chemistry, McOmie, ed., Plenum Press, NY, NY (1973); and, Protective Groups in Organic Synthesis, Greene, ed., John Wiley & Sons, NY, NY (1981) for the teaching of protective groups which may be useful in the preparation of compounds of the present invention.

10 By way of example only, useful amino-protecting groups may include, for example, C₁-C₁₀ alkanoyl groups such as formyl, acetyl, dichloroacetyl, propionyl, hexanoyl, 3,3-diethylhexanoyl, γ -chlorobutyl, and the like; C₁-C₁₀ alkoxycarbonyl and C₅-C₁₅ aryloxycarbonyl groups such as tert-butoxycarbonyl,

15 benzyloxycarbonyl, allyloxycarbonyl, 4-nitrobenzyloxycarbonyl, fluorenylmethyloxycarbonyl and cinnamoyloxycarbonyl; halo-(C₁-C₁₀)-alkoxycarbonyl such as 2,2,2-trichloroethoxycarbonyl; and C₁-C₁₅ arylalkyl and alkenyl group such as benzyl, phenethyl, allyl, trityl, and the like. Other commonly used amino-protecting groups

20 are those in the form of enamines prepared with β -keto-esters such as methyl or ethyl acetoacetate.

Useful carboxy-protecting groups may include, for example, C₁-C₁₀ alkyl groups such as methyl, tert-butyl, decyl; halo-C₁-C₁₀ alkyl such as 2,2,2-trichloroethyl, and 2-iodoethyl;

25 C₅-C₁₅ arylalkyl such as benzyl, 4-methoxybenzyl, 4-nitrobenzyl, triphenylmethyl, diphenylmethyl; C₁-C₁₀ alkanoyloxymethyl such as acetoxymethyl, propionoxymethyl and the like; and groups such as phenacyl, 4-halophenacyl, allyl, dimethylallyl, tri-(C₁-C₃ alkyl)silyl, such as trimethylsilyl, β -p-toluenesulfonylethyl, β -p-nitrophenylthio-

30 ethyl, 2,4,6-trimethylbenzyl, β -methylthioethyl, phthalimidomethyl, 2,4-dinitro-phenylsulphenyl, 2-nitrobenzhydryl and related groups.

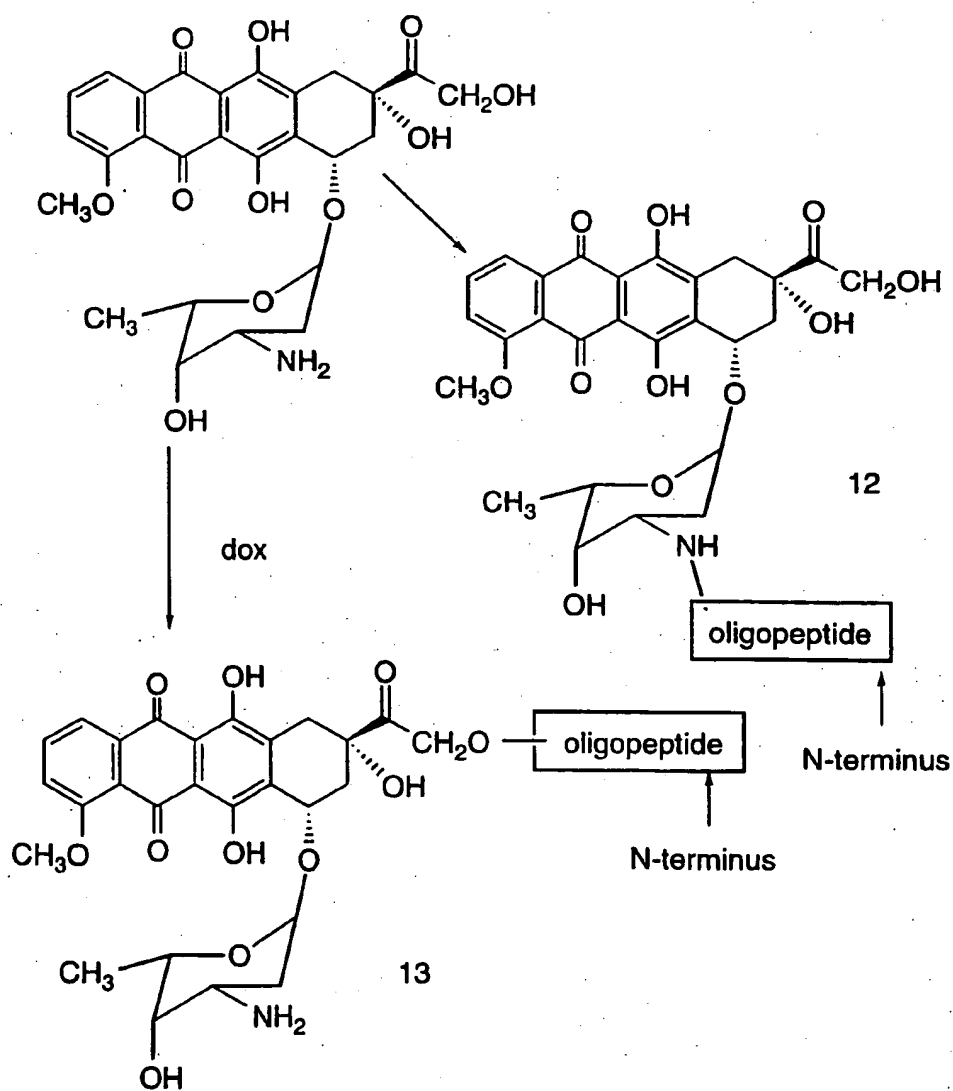
Similarly, useful hydroxy protecting groups may include, for example, the formyl group, the chloroacetyl group, the benzyl group, the benzhydryl group, the trityl group, the 4-nitrobenzyl group,

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the trimethylsilyl group, the phenacyl group, the tert-butyl group, the methoxymethyl group, the tetrahydropyranyl group, and the like.

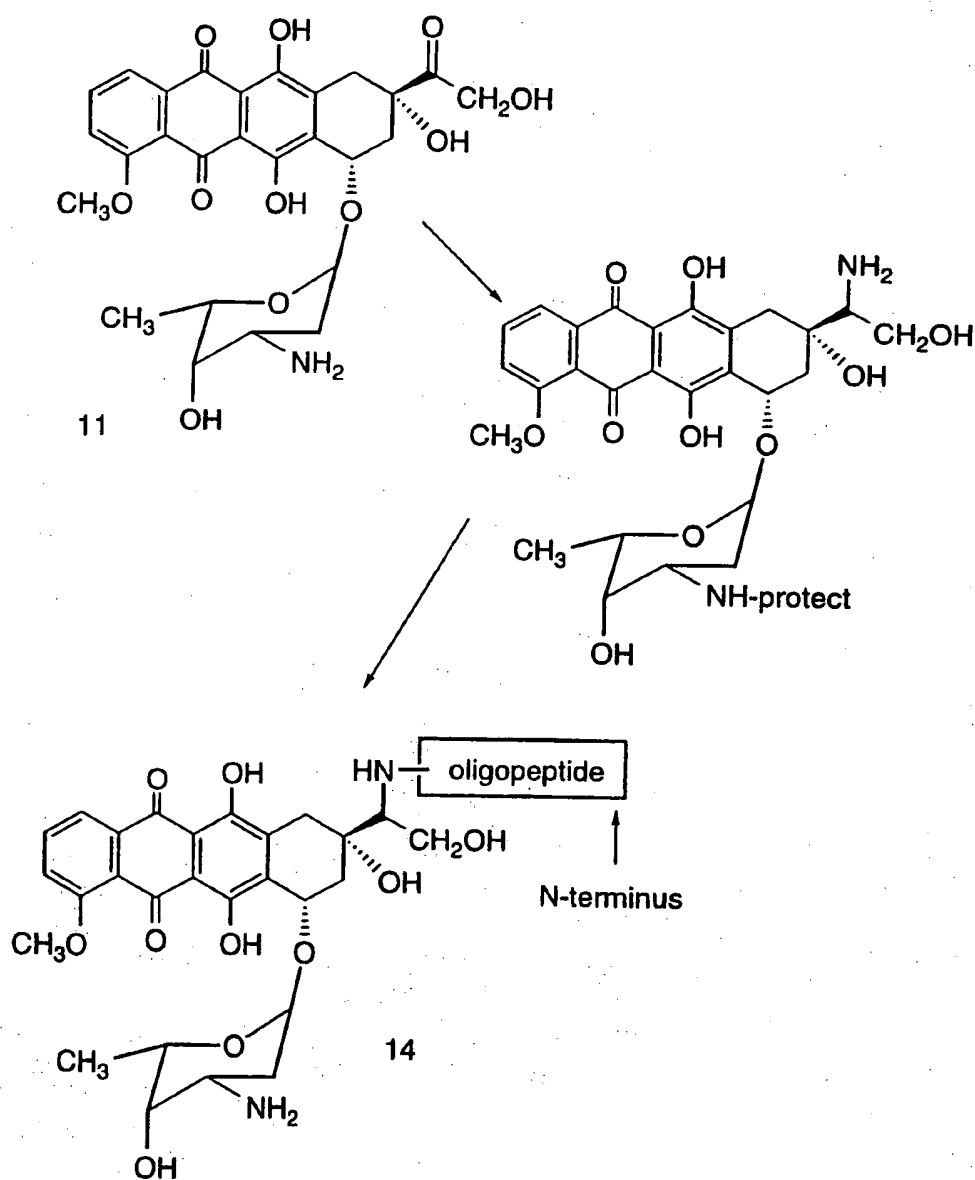
- 5 With respect to the preferred embodiment of an oligopeptide combined with the anthracycline antibiotic doxorubicin, the following Reaction Schemes illustrate the synthesis of the conjugates of the instant invention.

REACTION SCHEME I



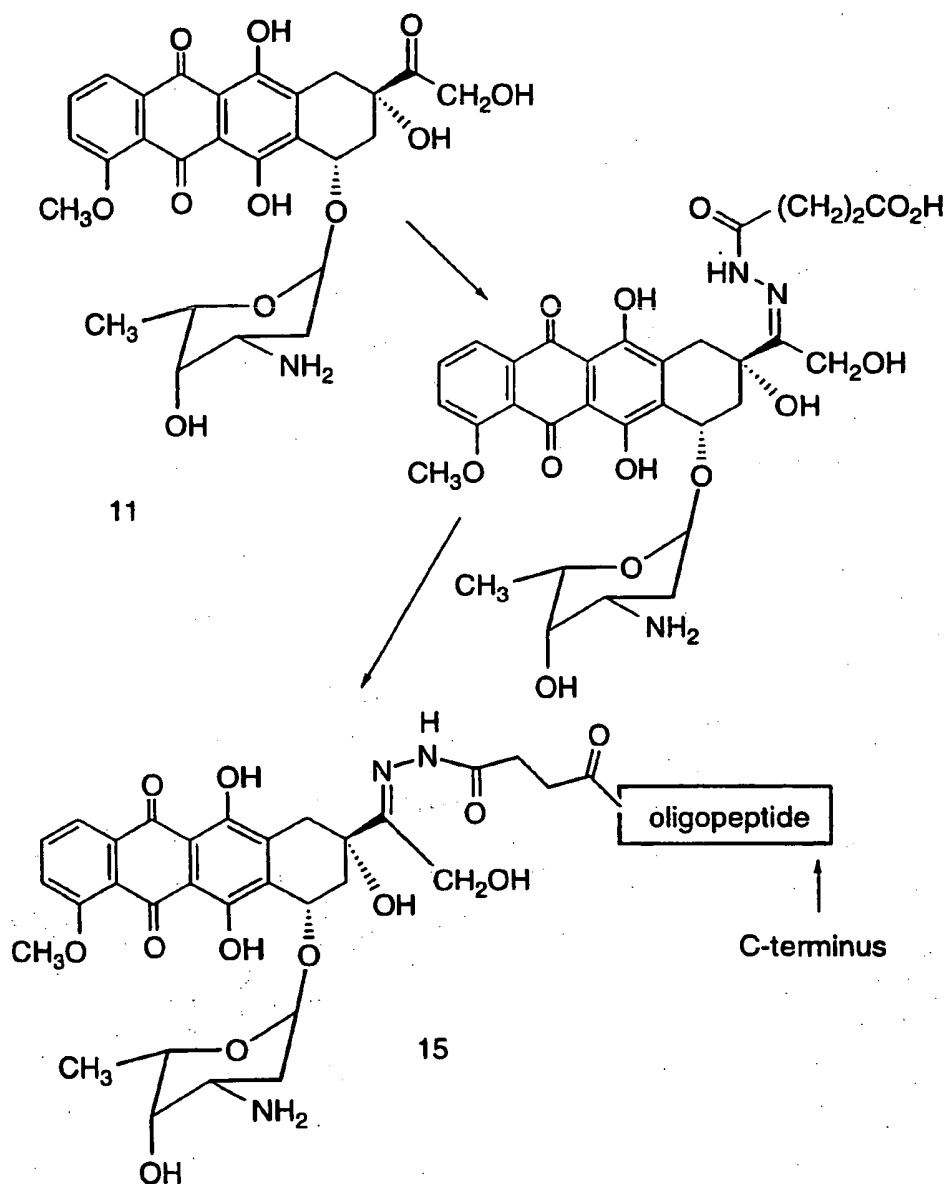
- 44 -

REACTION SCHEME II



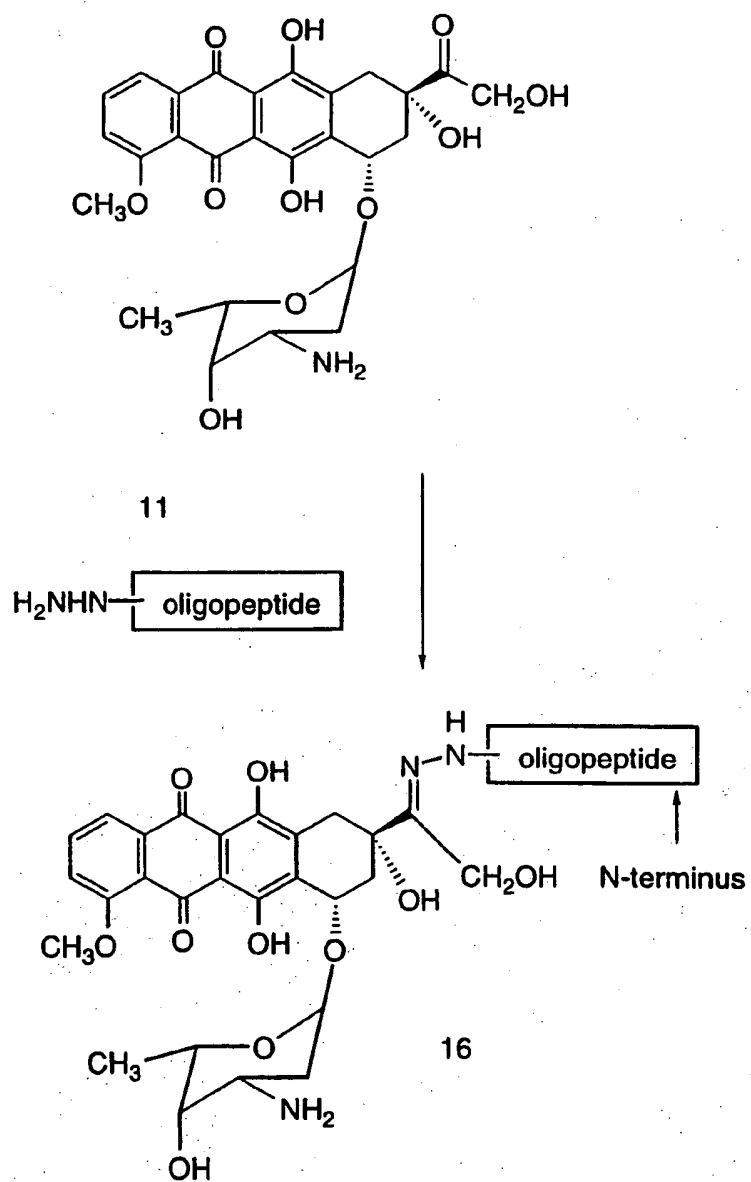
- 45 -

REACTION SCHEME III



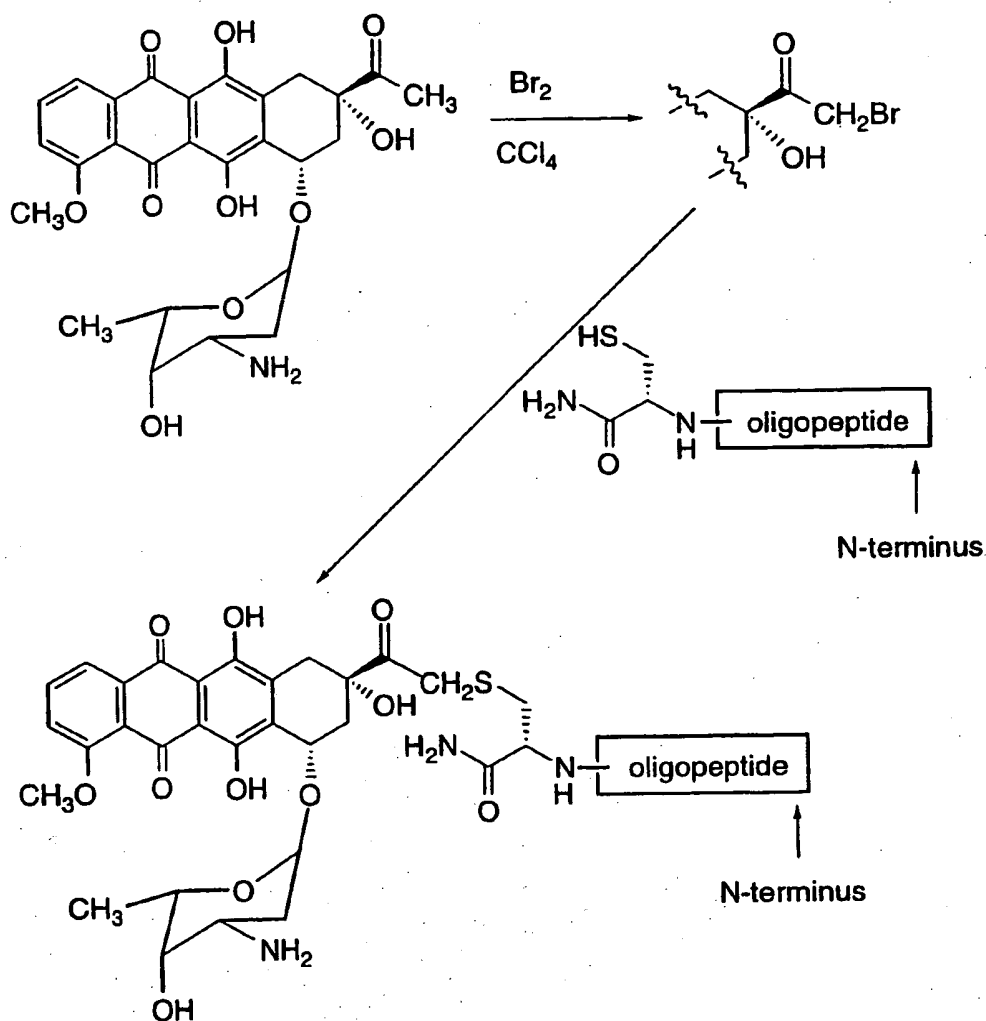
- 46 -

REACTION SCHEME IV



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REACTION SCHEME V



Reaction Scheme VI illustrates preparation of conjugates utilized in the instant method of treatment wherein the oligopeptides are combined with the vinca alkaloid cytotoxic agent vinblastine. Attachment of the N-terminus of the oligopeptide to vinblastine is illustrated (S.P. Kandukuri et al. J. Med. Chem. 28:1079-1088 (1985)).

Reaction Scheme VII illustrates preparation of conjugates of the oligopeptides of the instant invention and the vinca alkaloid

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cytotoxic agent vinblastine wherein the attachment of vinblastine is at the C-terminus of the oligopeptide. The use of the 1,3-diaminopropane linker is illustrative only; other spacer units between the carbonyl of vinblastine and the C-terminus of the oligopeptide are also envisioned.

5 Furthermore, Scheme VII illustrates a synthesis of conjugates wherein the C-4-position hydroxy moiety is reacetylated following the addition of the linker unit. Applicants have discovered that the desacetyl vinblastine conjugate is also efficacious and may be prepared by eliminating the steps shown in Reaction Scheme VII of protecting the

10 primary amine of the linker and reacting the intermediate with acetic anhydride, followed by deprotection of the amine. Conjugation of the oligopeptide at other positions and functional groups of vinblastine may be readily accomplished by one of ordinary skill in the art and is also expected to provide compounds useful in the treatment of prostate

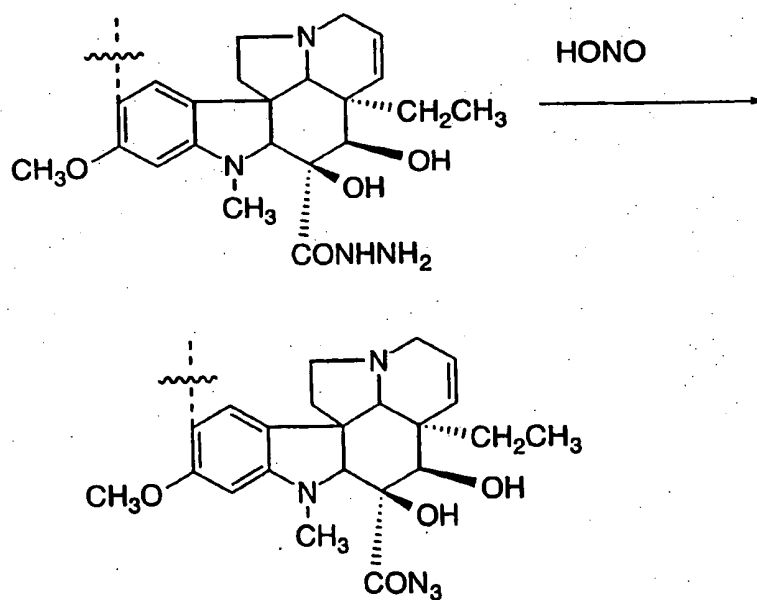
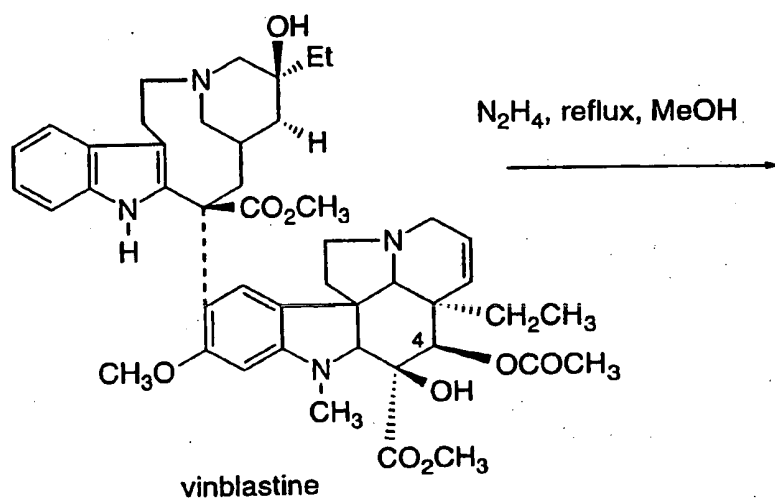
15 cancer.

It is also understood that conjugates may be prepared wherein the N-terminus of the oligopeptide, which comprises a cyclic amino acid having a hydrophilic substituent, utilized in the instant method of treatment is combined with one cytotoxic agent, such as

20 vinblastine, while the C-terminus is simultaneously attached to another cytotoxic agent, which is the same or different cytotoxic agent, such as doxorubicin. Reaction Scheme VIII illustrates the synthesis of such a polycytotoxic agent conjugate. Such a polycytotoxic conjugate may offer advantages over a conjugate containing only one cytotoxic agent.

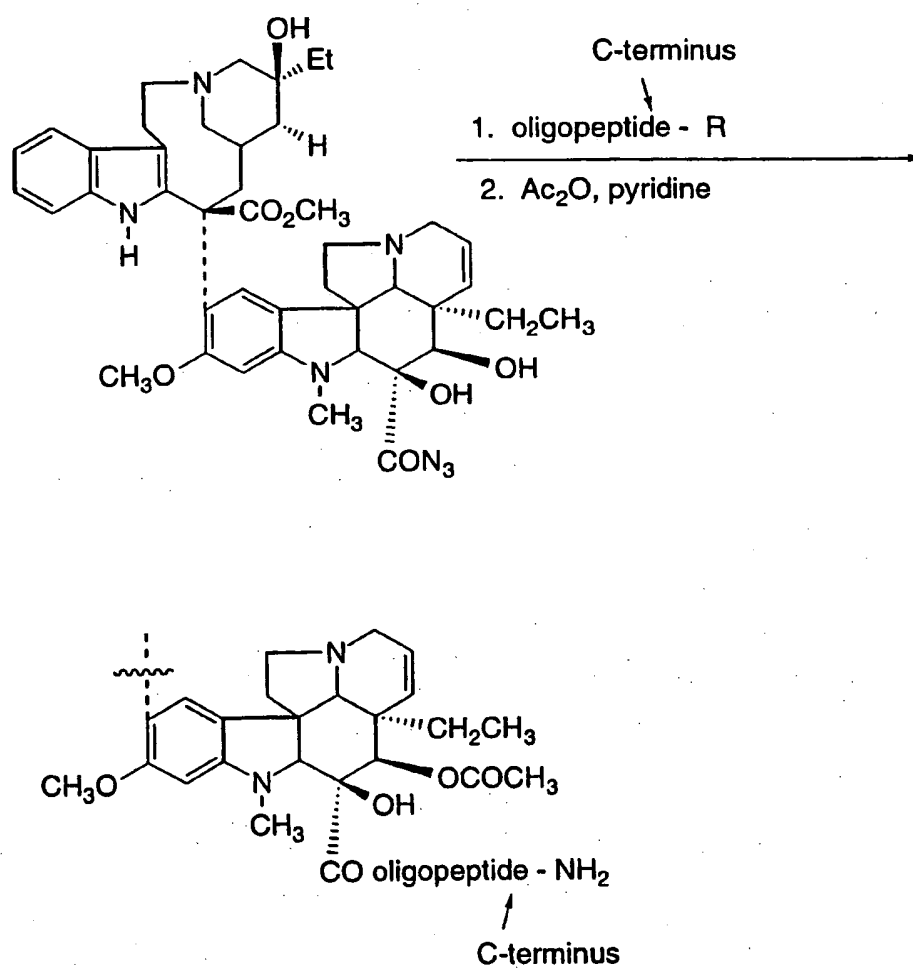
- 49 -

REACTION SCHEME VI



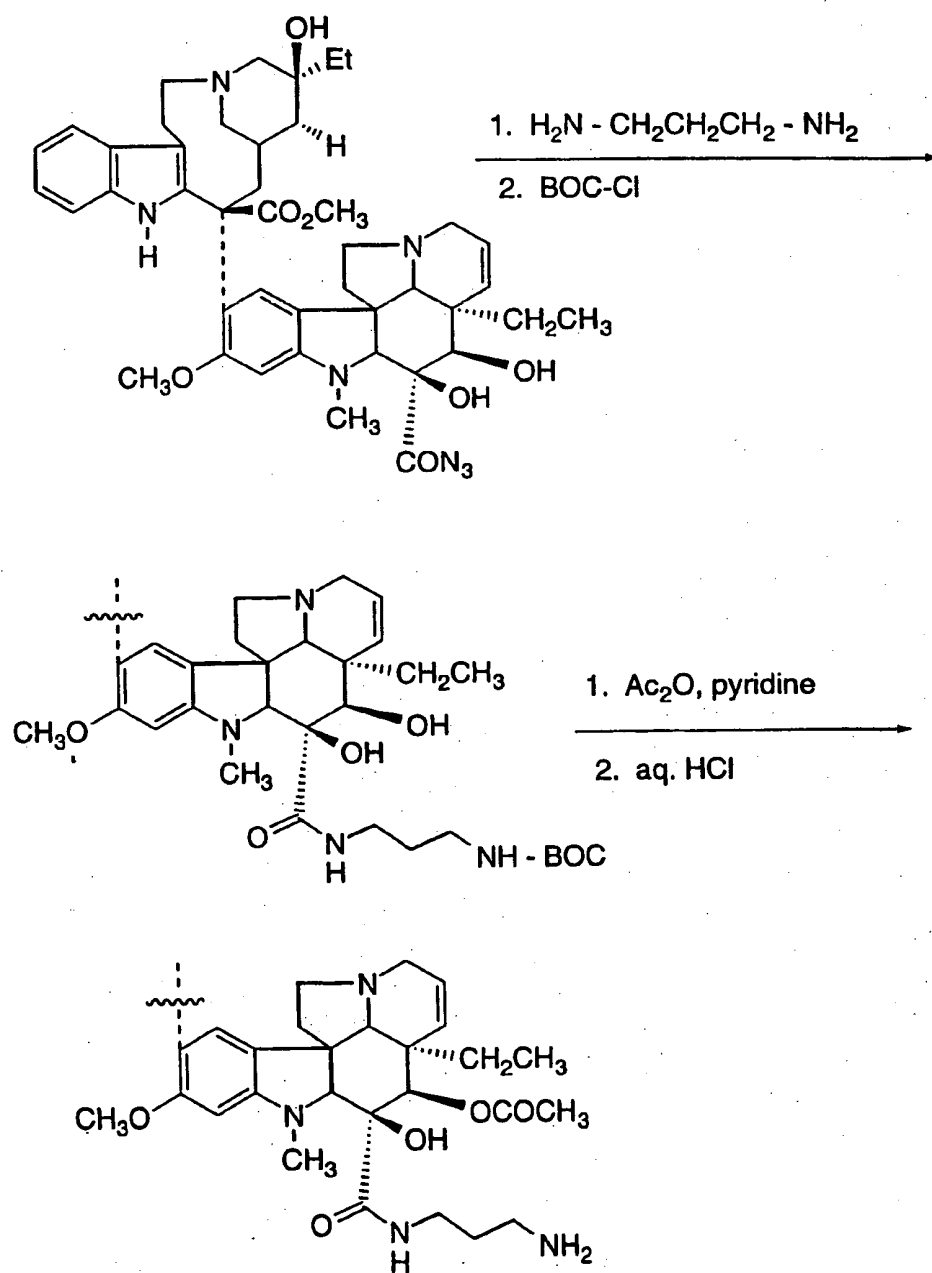
- 50 -

REACTION SCHEME VI (Continued)

wherein R is $-\text{NH}_2$, $-\text{O-alkyl}$ and the like

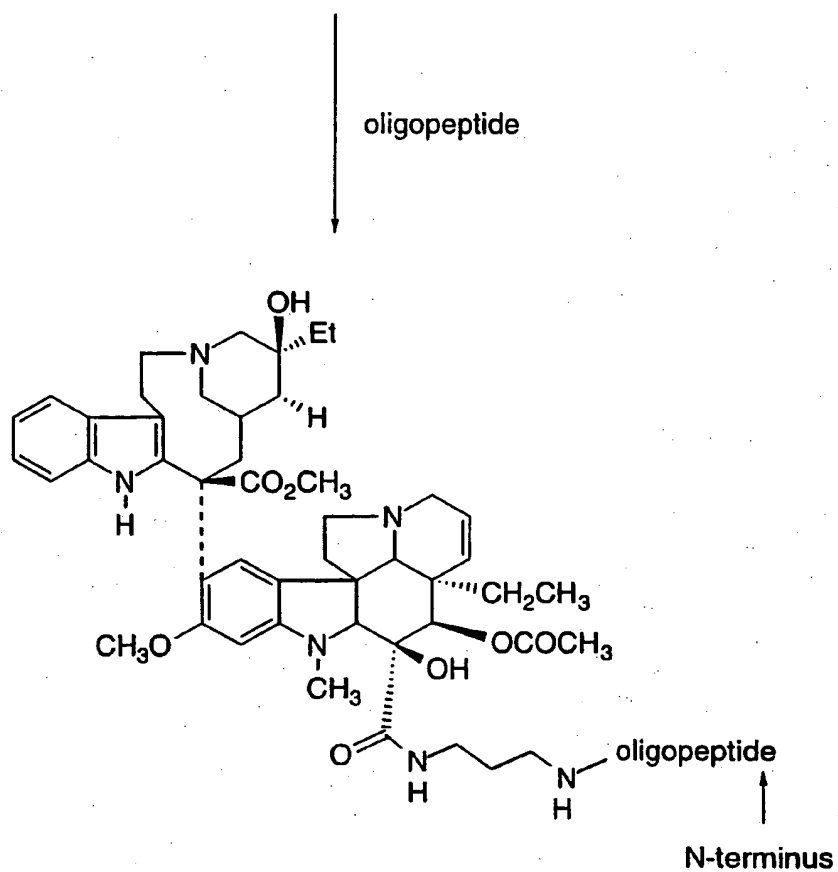
- 51 -

REACTION SCHEME VII



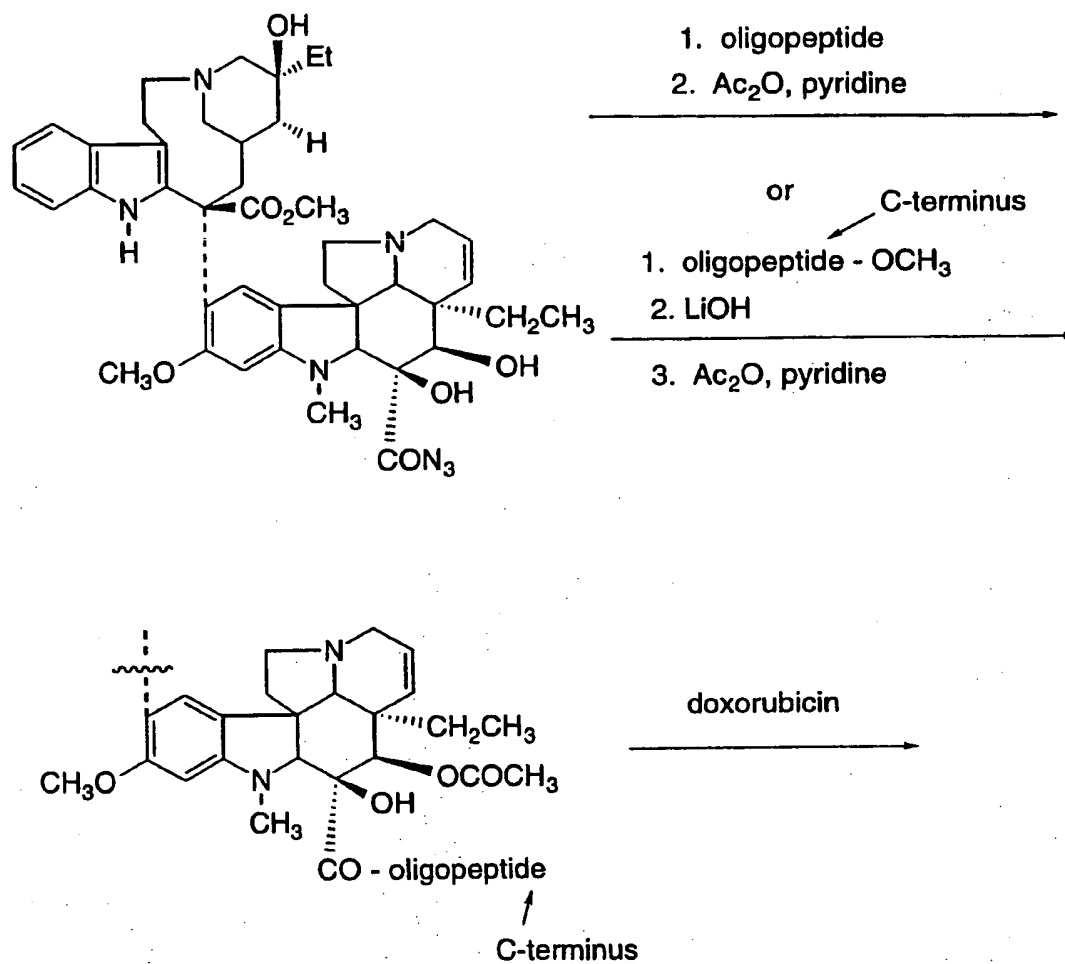
- 52 -

REACTION SCHEME VII (Continued)



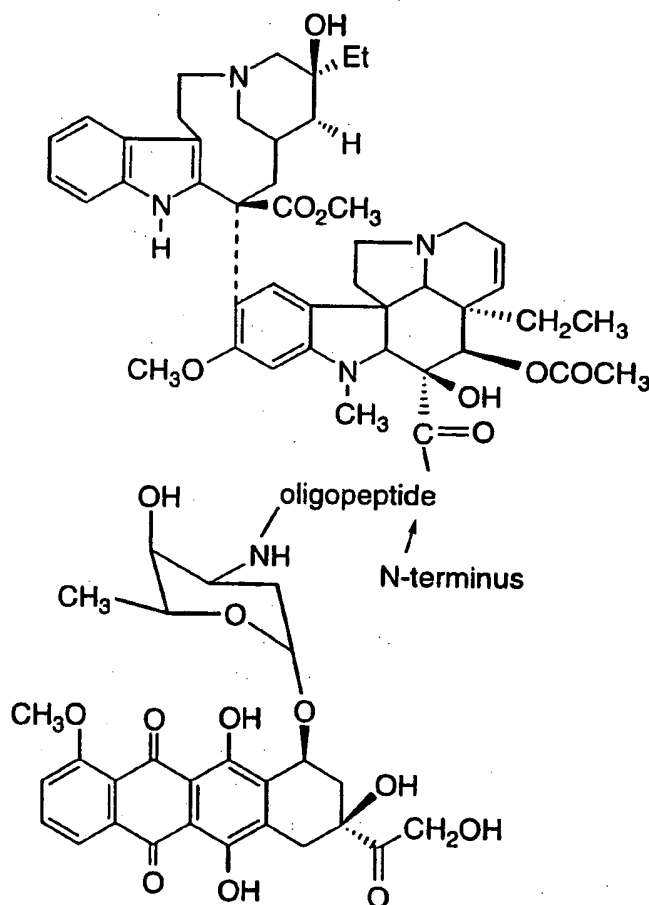
- 53 -

REACTION SCHEME VIII



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REACTION SCHEME VIII (Continued)



- 5 The oligopeptide-cytotoxic agent conjugates of the invention are administered to the patient in the form of a pharmaceutical composition which comprises a conjugate of of the instant invention and a pharmaceutically acceptable carrier, excipient or diluent therefor. As used, "pharmaceutically acceptable" refers to those agents which are
- 10 useful in the treatment or diagnosis of a warm-blooded animal including, for example, a human, equine, porcine, bovine, murine, canine, feline, or other mammal, as well as an avian or other warm-blooded

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animal. The preferred mode of administration is parenterally, particularly by the intravenous, intramuscular, subcutaneous, intra-peritoneal, or intralymphatic route. Such formulations can be prepared using carriers, diluents or excipients familiar to one skilled in the art.

- 5 In this regard, See, e.g. Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Company, edited by Osol et al. Such compositions may include proteins, such as serum proteins, for example, human serum albumin, buffers or buffering substances such as phosphates, other salts, or electrolytes, and the like. Suitable diluents may include,
10 for example, sterile water, isotonic saline, dilute aqueous dextrose, a polyhydric alcohol or mixtures of such alcohols, for example, glycerin, propylene glycol, polyethylene glycol and the like. The compositions may contain preservatives such as phenethyl alcohol, methyl and propyl parabens, thimerosal, and the like. If desired, the composition can
15 include about 0.05 to about 0.20 percent by weight of an antioxidant such as sodium metabisulfite or sodium bisulfite.

- As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as any product which results, directly or indirectly,
20 from combination of the specific ingredients in the specified amounts.

- For intravenous administration, the composition preferably will be prepared so that the amount administered to the patient will be from about 0.01 to about 1 g of the conjugate. Preferably, the amount administered will be in the range of about 0.2 g to about 1 g of the
25 conjugate. The conjugates of the invention are effective over a wide dosage range depending on factors such as the disease state to be treated or the biological effect to be modified, the manner in which the conjugate is administered, the age, weight and condition of the patient as well as other factors to be determined by the treating physician. Thus, the
30 amount administered to any given patient must be determined on an individual basis.

One skilled in the art will appreciate that although specific reagents and reaction conditions are outlined in the following examples, modification can be made which are meant to be encompassed by the

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spirit and scope of the invention. The following preparations and examples, therefore, are provided to further illustrate the invention, and are not limiting.

5

EXAMPLES

EXAMPLE 1

10 Preparation of Oligopeptides which Comprise the PSA Mediated Cleavage Site:

Blocked oligopeptides were prepared by solid-phase synthesis, using a double coupling protocol for the introduction of amino acids on the Applied Biosystems model 430A automated peptide synthesizer. Deprotection and removal of the oligopeptide from
15 the resin support were achieved by treatment with liquid hydrofluoric acid. The oligopeptides were purified by preparative high pressure liquid chromatography on reverse phase C18 silica columns using an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient. Identity and homogeneity of the oligopeptides were confirmed by amino acid
20 composition analysis, high pressure liquid chromatography, and fast atom bombardment mass spectral analysis. The oligopeptides that were prepared by this method are shown in Table 2.

TABLE 2

25

SEQ.ID.NO.	PEPTIDE / PEPTIDE-DOX CONJUGATE	Time to 50% Substrate, Cleavage by PSA (Min)
7 3	Ac-PSSChgQ-SV-acid	120
7 4	Ac-PASChgQ-SL-acid	150
7 5	Ac-(Dehydro-Pro)-ASChgQ-SL-acid	3 HOURS = 28%
6 8	Ac-(4-trans-L-Hyp)ASChgQ-SL-acid	75
7 6	Ac-(4-trans-L-Hyp)ChgQ-SSSL-acid	3 HOURS = 0% n=2
7 7	Ac-(4-trans-L-Hyp)ASChgQ-SThi-acid	20
7 8	Ac-(4-trans-L-Hyp)ASChgQ-S(TIC)-acid	3 HOURS = 16%
6 8	PEG(2)-(4-trans-L-Hyp)-ASChgQ-SL-acid	3 HOURS = 44%

4-trans-L-Hyp is *trans*-4-hydroxy-L-proline

Dehydro-Pro is 3,4-dehydro-L-proline

EXAMPLE 2

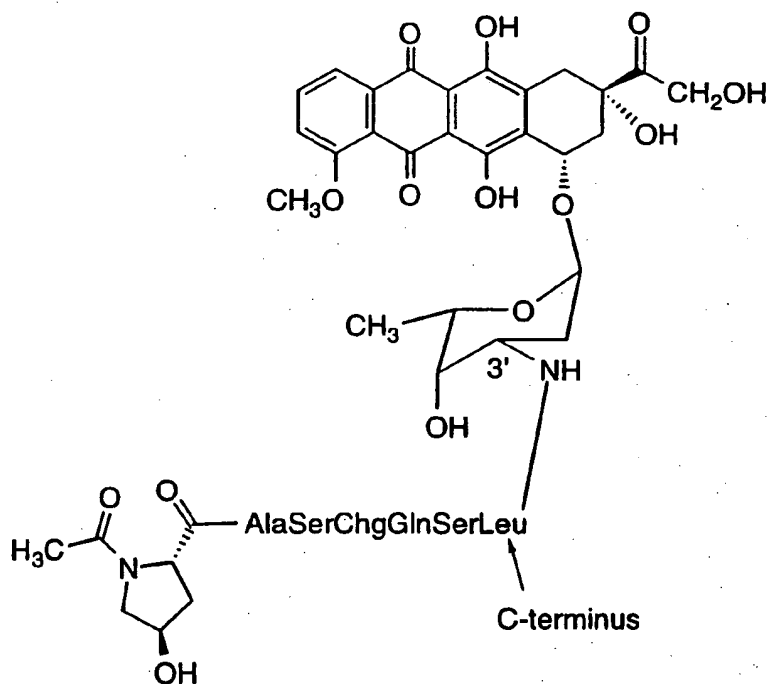
Assessment of the Recognition of Oligopeptides by Free PSA :

- 5 The oligopeptides prepared as described in Example 1 were individually dissolved in PSA digestion buffer (12 mM tris(hydroxymethyl)-aminomethane pH8.0, 25 mM NaCl, 0.5 mM CaCl₂) and the solution added to PSA at a molar ratio of 100 to 1. Alternatively, the PSA digestion buffer utilized is 50 mM tris(hydroxymethyl)-aminomethane
- 10 pH7.4, 140 mM NaCl. The reaction is quenched after various reaction times by the addition of trifluoroacetic acid (TFA) to a final 1% (volume/volume). Alternatively the reaction is quenched with 10mM ZnCl₂. The quenched reaction was analyzed by HPLC on a reversed-phase C18 column using an aqueous 0.1%TFA/acetonitrile gradient.
- 15 The results of the assessment are shown in Table 2. Table 2 shows the amount of time (in minutes) required for 50% cleavage of the noted oligopeptides with enzymatically active free PSA. Oligopeptides containing free amine moieties (ie. comprising hArg, Orn, Lys and or 3PAL) were tested as TFA salts. All other oligopeptides were tested as
- 20 neutral compounds.

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EXAMPLE 3

Preparation of [N-Ac-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox₁
(SEQ.ID.NO.: 68)



Step A: [N-Ac-(4-trans-L-Hyp(Bzl))]-Ala-Ser(Bzl)Chg-Gln-Ser(Bzl)Leu-PAM Resin (3-1).

- Starting with 0.5 mmol (0.67g) Boc-Leu-PAM resin, the
- 10 protected peptide was synthesized on a 430A ABI peptide synthesizer. The protocol used a 4 fold excess (2 mmol) of each of the following protected amino acids: Boc-Ser(Bzl), Boc-Gln, Boc-Chg, Boc-Ala, N-Boc-(4-trans-L-Hyp(Bzl)). Coupling was achieved using DCC and HOBT activation in methyl-2-pyrrolidinone. Acetic acid was used for
- 15 the introduction of the N terminal acetyl group. Removal of the Boc group was performed using 50% TFA in methylene chloride and the TFA salt neutralized with diisopropylethylamine. At the completion of the synthesis the peptide resin was dried to yield Intermediate 3-1.

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Step B: [N-Ac-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-OH
 (3-2)

5 The protected peptide resin (3-1), 1.2 g, was treated with HF (20 ml) for 1 hr at 0°C in the presence of anisole (2 ml). After evaporation of the HF, the residue was washed with ether, filtered and extracted with H₂O (200 ml). The filtrate was lyophilized to yield Intermediate 3-2.

10 **Step C:** [N-Ac-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox

 The above described intermediate (3-2), 1.157 g (1.45 mmol) was dissolved in DMSO (30 ml) and diluted with DMF (30 ml). To the solution was added doxorubicin hydrochloride, 516 mg (0.89 mmol) followed by 0.310 ml of diisopropylethylamine (1.78 mmol).
15 The stirred solution was cooled (0°C) and 0.276 ml of diphenylphosphoryl azide (1.28 mmol) added. After 30 minutes, an additional 0.276 ml (1.28 mmol) of DPPA was added and the pH adjusted to ~7.5 (pH paper) with diisopropylethylamine (DIEA). The pH of the cooled reaction (0°C) was maintained at ~7.5 with DIEA for the next 3 hrs.
20 and the reaction stirred at 0-4°C overnight. After 18 hrs., the reaction (found to be complete by analytical HPLC, system A) was concentrated to an oil. Purification of the crude product was achieved by preparative HPLC, Buffer A = 0.1% NH₄OAc-H₂O; B=CH₃CN. The crude product was dissolved in 400 ml of 100% A buffer, filtered and purified on a
25 C-18 reverse phase HPLC radial compression column (Waters, Delta-Pak, 15µM, 100Å). A step gradient of 100% A to 60% A was used at a flow rate of 75 ml/min (UV = 214nm). Homogeneous product fractions (evaluated by HPLC, system A) were pooled and freeze-dried. The product was dissolved in H₂O (300 ml), filtered and freeze-dried to
30 provide the purified title compound.

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HPLC conditions, system A

Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over 45 min.
5 A=0.1% TFA/H₂O, B=0.1% TFA/Acetonitrile
Flow: 1.5 ml/min.
Wavelength: 214 nM, 254 nM
Retention times: Doxorubicin = 13.66 min.
Ac-Hyp-Ala-Ser-Chg-Gln-Ser-Leu-OH = 10.8 min.
10 Ac-Hyp-Ala-Ser-Chg-Gln-Ser-Leu-Dox = 18.2 min.

PHYSICAL PROPERTIES

15 The physical/chemical properties of the product of Step C are shown below:

Molecular Formula: C₆₂H₈₅N₉O₂₃
Molecular Weight: 1323.6
High Resolution ES Mass Spec: 1341.7 (NH₄⁺)
20 HPLC: System A
Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over
45 min. A=0.1% TFA/H₂O, B=0.1%
TFA/Acetonitrile
25 Flow: 1.5 ml/min.
Wavelength: 214 nm, 254 nm
Retention Time: 18.2 min.
Amino Acid Compositional Analysis¹:

	Theory	Found
30 Ala (1)		1.00
Ser (2)		1.88
Chg (1)		0.91
Gln ² (1)		1.00 (as Glu)
Hyp (1)		0.80

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Leu (1) 1.01
Peptide Content: 0.657 $\mu\text{mol/mg}$
Note: ¹20 hr., 100°C, 6N HCl
²Gln converted to Glu

5

Table 3 shows other peptide-doxorubicin conjugates that were prepared by the procedures described in Example 3, but utilizing the appropriate amino acid residues and blocking group acylation.

10

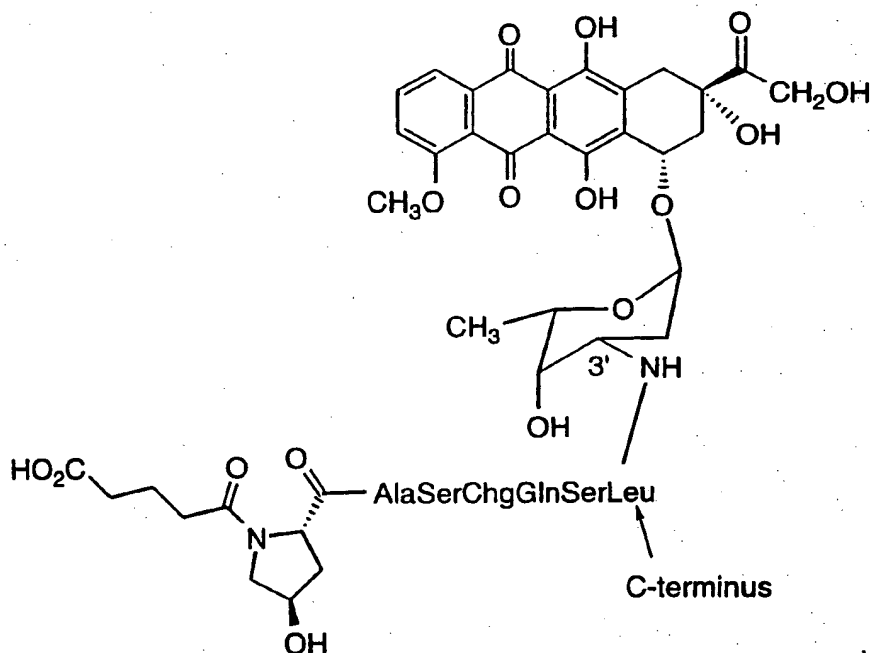
TABLE 3

<u>SEQ. ID.NO.</u>	<u>PEPTIDE / PEPTIDE-DOX CONJUGATE</u>	<u>Time to 50% Substrate Cleavage by PSA (Min)</u>
89	Ac-(4-trans-L-Hyp)ASChgQ-SThi-DOX (3')	INSOLUBLE
74	Ac-(4-trans-L-Hyp)ASChgQ-StBuAla-DOX (3')	25
73	PEG(2)-(4-trans-L-Hyp)ASChgQ-SL-DOX (3')	20
68	Ac-(4-trans-L-Hyp)ASChgQ-SL-DOX (3')	20

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EXAMPLE 4

Preparation of [N-Glutaryl-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox (SEQ.ID.NO.: 71)



5

Step A: [N-Glutaryl(OFm)-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-PAM Resin

- Starting with 0.5mmol (0.67g) Boc-Leu-PAM resin, the
- 10 protected peptide was synthesized on a 430A ABI peptide synthesizer. The protocol used a 4 fold excess (2 mmol) of each of the following protected amino acids: Fmoc-Ser(tBu), Fmoc-Gln(Trt), Fmoc-Chg, Fmoc-Ala, Boc-(4-trans-L-Hyp). Coupling was achieved using DCC and HOBT activation in methyl-2-pyrrolidinone. The intermediate
- 15 mono fluorenylmethyl ester of glutaric acid [Glutaryl(OFm)] was used for the introduction of the N-terminal glutaryl group. Removal of the Fmoc group was performed using 20% piperidine. The acid sensitive protecting groups, Boc, Trt and tBu, were removed with 50% TFA in methylene chloride. Neutralization of the TFA salt was with

- 63 -

diisopropylethylamine. At the completion of the synthesis, the peptide resin was dried to yield the title compound.

Step B: [N-Glutaryl(OFm)-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-OH

5 The protected peptide resin from Step A, 1.2 g, was treated with HF (20 ml) for 1 hr at 0°C in the presence of anisole (2 ml). After evaporation of the HF, the residue was washed with ether, filtered and extracted with DMF. The DMF filtrate (75 ml) was concentrated to dryness and triturated with H₂O. The insoluble product was filtered and dried to provide the title compound.

Step C: [N-Glutaryl(OFm)-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox

15 The above prepared intermediate from Step B, (1.33g, 1.27mmol) was dissolved in DMSO (6 ml) and DMF (69 ml). To the solution was added doxorubicin hydrochloride, 599 mg (1.03 mmol) followed by 376µl of diisopropylethylamine (2.16 mmol). The stirred solution was cooled (0°C) and 324 µl of diphenylphosphoryl azide (1.5mmol) added. After 30 minutes, an additional 324 µl of DPPA was added and the pH adjusted to ~7.5 (pH paper) with diisopropylethylamine (DIEA). The pH of the cooled reaction (0°C) was maintained at ~7.5 with DIEA for the next 3 hrs and the reaction stirred at 0-4°C overnight. After 18 hrs., the reaction (found to be complete by analytical HPLC, system A) was concentrated to provide the title compound as an oil.

Step D: [N-Glutaryl-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox

30 The above product from Step C was dissolved in DMF (54 ml), cooled (0°C) and 14 ml of piperidine added. The solution was concentrated to dryness and purified by preparative HPLC. (A=0.1% NH₄OAc-H₂O; B=CH₃CN.) The crude product was dissolved in 100 ml of 80% A buffer, filtered and purified on a C-18 reverse phase HPLC

- 64 -

- radial compression column (Waters, Delta-Pak, 15 μ , 100Å). A step gradient of 80% A to 67% A was used at a flow rate of 75 ml/min (uv = 214nm). Homogeneous product fractions (evaluated by HPLC, system A) were pooled and freeze-dried. The product was further purified
- 5 using the above HPLC column. Buffer A = 15% acetic acid-H₂O; B=15% acetic acid-methanol. The product was dissolved in 100 ml of 20% B/80% A buffer and purified. A step gradient of 20% B to 80% B was used at a flow rate of 75 ml/min (uv = 260nm). Homogeneous product fractions (evaluated by HPLC, system A) were
- 10 pooled, concentrated and freeze-dried from H₂O to yield the purified title compound.

HPLC conditions, system A

- 15 Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over 45 min.
A=0.1% TFA/H₂O, B=0.1% TFA/Acetonitrile
Flow: 1.5 ml/min.
Wavelength: 214 nm, 254 nm
- 20 Retention times: Doxorubicin = 13.66 min.
[N-Glutaryl(OFm) - (4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-OH =
19.66 min.
[N-Glutaryl(OFm) - (4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox =
24.8 min.
- 25 [N-Glutaryl-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox =
18.3 min.

High Resolution ES Mass Spec: 1418.78 (Na⁺)

- HPLC: System A
- 30 Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over 45 min.
A=0.1% TFA/H₂O, B=0.1% TFA/Acetonitrile
Flow: 1.5 ml/min.
Wavelength: 214 nm, 254 nm
- 35 Retention Time: 18.3 min.

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Amino Acid Compositional Analysis¹:

	<u>Theory</u>	<u>Found</u>
	Ala(1)	0.99
5	Ser (2)	2.02
	Chg (1)	1.00
	Gln ² (1)	1.01 (as Glu)
	Hyp (1)	0.99
	Leu (1)	1.00
10	Peptide Content:	0.682 μ mol/mg
	Note: ¹ 20 hr., 100°C, 6N HCl	
	² Gln converted to Glu	

Table 4 shows other peptide-doxorubicin conjugates that were prepared by the procedures described in Example 4, but utilizing the appropriate amino acid residues and blocking group acylation.

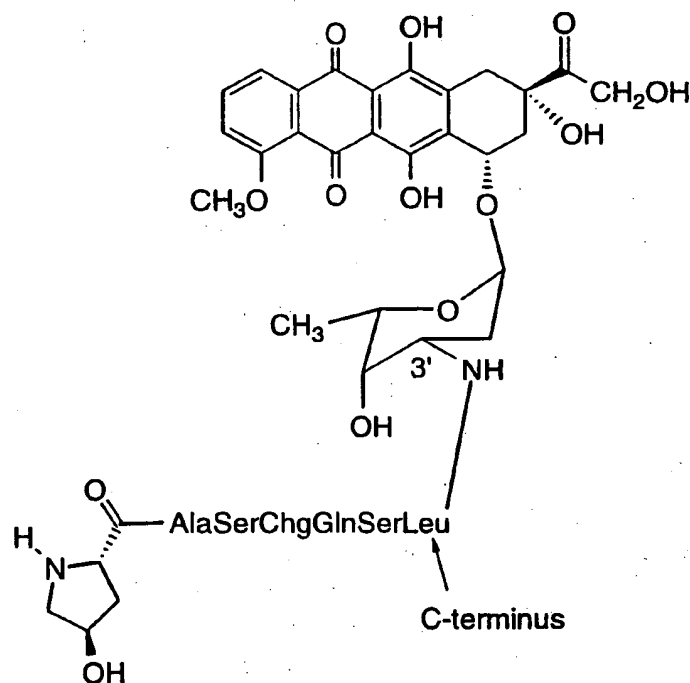
TABLE 4

	SEQ. ID. NO.
Succinyl-(4-trans-L-Hyp)ASChgQ-SV-DOX (3')	75
Glutaryl-(4-trans-L-Hyp)ASChgQ-SV-DOX (3')	76
Glutaryl-(4-trans-L-Hyp)ASChgQ-SI-DOX (3')	77
Succinyl-(4-trans-L-Hyp)SSChgQ-SI-DOX (3')	78
Succinyl-(4-trans-L-Hyp)ASChgQ-SI-DOX (3')	79
Succinyl-(4-trans-L-Hyp)ASChgQ-SAbu-DOX (3')	80
Glutaryl-(4-trans-L-Hyp)SSChgQ-SI-DOX (3')	81
Glutaryl-(4-trans-L-Hyp)SSChgQ-SL-DOX (3')	82
PEG(2)-(4-trans-L-Hyp)SSChgQ-SL-DOX (3')	83
Succinyl-(4-trans-L-Hyp)ASChgQ-SThi-DOX (3')	84
PEG(4)-(4-trans-L-Hyp)-SSChgQ-SL-DOX (3')	85
PEG(2)-(4-trans-L-Hyp)ASChgQ-SThi-DOX(3')	86
Succinyl-3,4-(diOH)PASChgQ-SL-DOX (3')	87
Malonyl-(4-trans-L-Hyp)ASChgQ-SL-DOX (3')	88

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EXAMPLE 5

Preparation of (4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-Dox
(SEQ.ID.NO.: 70)



5

Step A: Fmoc-(4-trans-L-Hyp(Bzl))-Ala-Ser(Bzl)Chg-Gln-Ser(Bzl)Leu-PAM Resin

- Starting with 0.5 mmol (0.67g) Boc-Leu-PAM resin, the
- 10 protected peptide was synthesized on a 430A ABI peptide synthesizer. The protocol used a 4 fold excess (2 mmol) of each of the following protected amino acids: Boc-Ser(Bzl), Boc-Gln, Boc-Chg, Boc-Ala, N-Boc-(4-trans-L-Hyp(Bzl)). Coupling was achieved using DCC and HOBT activation in methyl-2-pyrrolidinone. Fmoc-OSu (succinamidyl
- 15 ester of Fmoc) was used for the introduction of the N-terminal protecting group. Removal of the Boc group was performed using

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50% TFA in methylene chloride and the TFA salt neutralized with diisopropylethylamine. At the completion of the synthesis the peptide resin was dried to yield the title intermediate.

5 **Step B:** Fmoc-(4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-OH

The protected peptide resin from Step A, 1.1 g, was treated with HF (20 ml) for 1 hr at 0°C in the presence of anisole (2 ml). After evaporation of the HF, the residue was washed with ether, filtered and extracted with H₂O (200 ml). The filtrate was lyophilized to yield the
10 title intermediate.

Step C: Fmoc-(4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-Dox

The intermediate from Step B, 0.274 g, was dissolved in DMSO (10 ml) and diluted with DMF (10 ml). To the solution was
15 added doxorubicin hydrochloride, 104 mg followed by 62 µL of diisopropylethylamine. The stirred solution was cooled (0°C) and 56 µL of diphenylphosphoryl azide added. After 30 minutes, an additional 56 µL of DPPA was added and the pH adjusted to ~7.5 (pH paper) with diisopropylethylamine (DIEA). The pH of the cooled reaction (0°C)
20 was maintained at ~7.5 with DIEA. After 4 hrs., the reaction (found to be complete by analytical HPLC, system A) was concentrated to an oil. HPLC conditions, system A

Step D: (4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-Dox

25 The above product from Step C was dissolved in DMF (10 ml), cooled (0°C) and 4 ml of piperidine added. The solution was concentrated to dryness and purified by preparative HPLC. (A=0.1% NH₄OAc-H₂O; B=CH₃CN.) The crude product was dissolved in 100 ml of 90% A buffer, filtered and purified on a C-18 reverse phase HPLC
30 radial compression column (Waters, Delta-Pak, 15µ, 100Å). A step gradient of 90% A to 65% A was used at a flow rate of 75 ml/min (uv = 214nm). Homogeneous product fractions (evaluated by HPLC, system A) were pooled and freeze-dried.

35 HPLC conditions, system A

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- Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over 45 min.
A=0.1% TFA/H₂O, B=0.1% TFA/Acetonitrile
- 5 Flow: 1.5 ml/min.
Wavelength: 214 nm, 254 nm
Retention times: Doxorubicin = 13.66 min.
Fmoc - (4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-OH = 18.6 min.
Fmoc - (4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-Dox = 23.8 min.
- 10 (4-trans-L-Hyp)-Ala-Ser-Chg-Gln-Ser-Leu-Dox = 17.6 min.
- Molecular Formula: C₆₀H₈₃N₉O₂₂
Molecular Weight: 1281.56
High Resolution ES Mass Spec: 1282.59 (MH⁺)
- 15 HPLC: System A
Column: Vydac 15 cm #218TP5415, C18
Eluant: Gradient 95:5 (A:B) to 5:95 (A:B) over 45 min.
A=0.1% TFA/H₂O, B=0.1% TFA/Acetonitrile
Flow: 1.5 ml/min.
- 20 Wavelength: 214 nm, 254 nm
Retention Time: 17.6 min.

Amino Acid Compositional Analysis¹:

	<u>Theory</u>	<u>Found</u>
25	Ala (1)	1.00
	Ser (2)	1.94
	Chg (1)	0.94
	Gln ² (1)	1.05 (as Glu)
	Hyp (1)	0.96
30	Leu (1)	1.03

Peptide Content: 0.690 µmol/mg

Note: ¹20 hr., 100°C, 6N HCl²Gln converted to Glu

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EXAMPLE 6Assessment of the Recognition of Oligopeptide-Doxorubicin Conjugates by Free PSA :

5

The conjugates prepared as described in Examples 3-5 were individually dissolved in PSA digestion buffer (12 mM tris(hydroxymethyl)-aminomethane pH8.0, 25 mM NaCl, 0.5 mM CaCl₂) and the solution added to PSA at a molar ration of 100 to 1. Alternatively, the PSA digestion buffer utilized is 50 mM tris(hydroxymethyl)-aminomethane pH7.4, 140 mM NaCl. The reaction is quenched after various reaction times by the addition of trifluoroacetic acid (TFA) to a final 1% (volume/volume). Alternatively the reaction is quenched with 10mM ZnCl₂. The quenched reaction was analyzed by HPLC on a reversed-phase C18 column using an aqueous 0.1%TFA/acetonitrile gradient. The results of the assessment are shown in Table 3. Table 3 shows the amount of time (in minutes) required for 50% cleavage of the noted oligopeptide-cytotoxic agent conjugates with enzymatically active free PSA. If no salt is indicated for the conjugate, the free conjugate was tested. The oligopeptide-cytotoxic agent conjugates described in Examples 4 and 5 were assessed for the amount of time (in minutes) required for 50% cleavage of the oligopeptide with enzymatically active free PSA and 50% cleavage occurred in less than 2 hours for those conjugates.

25

EXAMPLE 7In vitro Assay of Cytotoxicity of Peptidyl Derivatives of Doxorubicin:

The cytotoxicities of the cleaveable oligopeptide-doxorubicin conjugates, prepared as described in Examples 3 and 4, against a line of cells which is known to be killed by unmodified doxorubicin were assessed with an Alamar Blue assay. Specifically, cell cultures of LNCap prostate tumor cells (which express enzymatically active PSA) or DuPRO cells in 96 well plates was diluted with medium

30

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(Dulbecco's Minimum Essential Medium- α [MEM- α]) containing various concentrations of a given conjugate (final plate well volume of 200 μ l). The cells were incubated for 3 days at 37°C, 20 μ l of Alamar Blue is added to the assay well. The cells were further incubated and the assay plates were read on a EL-310 ELISA reader at the dual wavelengths of 570 and 600 nm at 4 and 7 hours after addition of Alamar Blue. Relative percentage viability at the various concentration of conjugate tested was then calculated versus control (no conjugate) cultures. Some results of this assessment are shown in Table 5. If no salt is indicated, the free conjugate was tested.

TABLE 5

<u>SEQ.</u> <u>ID.NO.</u>	<u>PEPTIDE / PEPTIDE-DOX CONJUGATE</u>	<u>LNCaP Cell Kill in</u> <u>72 HRS</u> <u>EC 50 (μM)</u>
74	Ac-(4-trans-L-Hyp)ASChgQ-StBuAla-DOX (3')	100 (DuPRO > 100) n = 2
68	Ac-(4-trans-L-Hyp)ASChgQ-SL-DOX (3')	4.5 (DuPRO = 90)

15

EXAMPLE 8In vivo Efficacy of Peptidyl -Cytotoxic Agent Conjugates

LNCaP.FGC or DuPRO-1 cells are trypsinized, resuspended in the growth medium and centrifuged for 6 mins. at 200xg. The cells are resuspended in serum-free MEM- α and counted. The appropriate volume of this solution containing the desired number of cells is then transferred to a conical centrifuge tube, centrifuged as before and resuspended in the appropriate volume of a cold 1:1 mixture of MEM- α Matrigel (Collaborative Biomedical Products, New Bedford, Mass.). The suspension is kept on ice until the animals are inoculated.

Harlan Sprague Dawley male nude mice (10-12 weeks old) are restrained without anesthesia and are inoculated with 0.5 mL of cell suspension on the left flank by subcutaneous injection using a 22G

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needle. Mice are either given approximately 5×10^5 DuPRO cells or 1.5×10^7 LNCaP.FGC cells.

5 Following inoculation with the tumor cells the mice are treated under one of two protocols:

Protocol A:

10 One day after cell inoculation the animals are dosed by interperitoneal administration with a 0.1-0.5 mL volume of test conjugate, doxorubicin or vehicle control (sterile water). Dosages of the conjugate and doxorubicin are initially the maximum non-lethal amount, but may be subsequently titrated lower. Identical doses are administered at 24 hour intervals for 5 days. After 10 days, blood samples are removed from the mice and the serum level of PSA is
15 determined. Similar serum PSA levels are determined at 5-10 day intervals. At the end of 5.5 weeks the mice are sacrificed and weights of any tumors present are measured and serum PSA again determined. The animals' weights are determined at the beginning and end of the assay.

20

Protocol B:

Ten days after cell inoculation, blood samples are removed from the animals and serum levels of PSA are determined. Animals are then grouped according to their PSA serum levels. At 14-15 days after
25 cell inoculation, the animals are dosed by interperitoneal administration with a 0.1-0.5 mL volume of test conjugate, doxorubicin or vehicle control (sterile water). Dosages of the conjugate and doxorubicin are initially the maximum non-lethal amount, but may be subsequently titrated lower. Identical doses are administered at 24 hour intervals for
30 5 days. Serum PSA levels are determined at 5-10 day intervals. At the end of 5.5 weeks the mice are sacrificed, weights of any tumors present are measured and serum PSA again determined. The animals' weights are determined at the beginning and end of the assay.

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Protocol C:

One day after cell inoculation, the animals are dosed by interperitoneal administration with a 0.1-0.5 mL volume of test conjugate, doxorubicin or vehicle control (sterile water). Dosages of the conjugate and doxorubicin are initially the maximum non-lethal amount, but may be subsequently titrated lower. Identical doses are administered at 7 day intervals for 5 consecutive weeks. Serum PSA levels are determined immediately prior to or at the time of sacrificing the mice. At the end of 5.5 weeks the mice are sacrificed and weights of any tumors present are measured. The animals' weights are determined at the beginning and end of the assay.

EXAMPLE 9

15 *In vitro* determination of proteolytic cleavage of conjugates by endogenous non-PSA proteases

Step A: Preparation of proteolytic tissue extracts

20 All procedures are carried out at 4°C. Appropriate animals are sacrificed and the relevant tissues are isolated and stored in liquid nitrogen. The frozen tissue is pulverized using a mortar and pestle and the pulverized tissue is transferred to a Potter-Elvehjem homogenizer and 2 volumes of Buffer A (50 mM Tris containing 1.15% KCl, pH 7.5) are added. The tissue is then disrupted with 20 strokes using first a loose fitting and then a tight fitting pestle. The homogenate is centrifuged at 25 10,000 x g in a swinging bucket rotor (HB4-5), the pellet is discarded and the supernatant centrifuged at 100,000 x g (Ti 70). The supernatant (cytosol) is saved.

30 The pellet is resuspended in Buffer B (10 mM EDTA containing 1.15% KCl, pH 7.5) using the same volume as used above with Buffer A. The suspension is homogenized in a dounce homogenizer and the solution centrifuged at 100,000x g. The supernatant is discarded and the pellet (membrane) resuspended in Buffer C (10 mM potassium phosphate buffer containing 0.25 M

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sucrose, pH 7.4), using 1/2 the volume used above, and homogenized with a dounce homogenizer.

Protein content of the two solutions (cytosol and membrane) is determine using the Bradford assay. Assay aliquots are
5 then removed and frozen in liquid N₂. The aliquots are stored at -70° C.

Step B: Proteolytic cleavage assay

For each time point, 20 microgram of peptide-doxorubicin conjugate and 150 micrograms of tissue protein, prepared as described
10 in Step A and as determined by Bradford in reaction buffer are placed in solution of final volume of 200 microliters in buffer (50 mM TRIS, 140 mM NaCl, pH 7.2). Assay reactions are run for 0, 30, 60, 120, and 180 minutes and are then quenched immediately in boiling water for 90 seconds. Reaction products are analyzed by HPLC using a VYDAC C18
15 15 cm column in water / acetonitrile (5% to 50% acetonitrile over 30 minutes).

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SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION
- (i) APPLICANT: Garsky, Victor M.
Feng, Dong-Mei
DeFeo-Jones, Deborah
- 10 (ii) TITLE OF THE INVENTION: CONJUGATES USEFUL IN THE
TREATMENT
OF PROSTATE CANCER
- 15 (iii) NUMBER OF SEQUENCES: 97
- (iv) CORRESPONDENCE ADDRESS:
20 (A) ADDRESSEE: Merck & Co., Inc.
(B) STREET: P.O. Box 2000, 126 E. Lincoln Ave.
(C) CITY: Rahway
(D) STATE: NJ
(E) COUNTRY: USA
(F) ZIP: 07065-0900
- 25 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
30 (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
35 (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/029,224
(B) FILING DATE: 30-OCT-1996
- 40 (A) APPLICATION NUMBER: 60/042,921
(B) FILING DATE: 04-APR-1997
- 45 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Muthard, David A
(B) REGISTRATION NUMBER: 35,297
(C) REFERENCE/DOCKET NUMBER: 19821Y
- 50 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 908-594-3903
(B) TELEFAX: 908-594-4720
(C) TELEX:
- 55 (2) INFORMATION FOR SEQ ID NO:1:

- 75 -

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 10 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
Xaa Xaa Ser Tyr Gln Ser Ser
1 5
- 20 (2) INFORMATION FOR SEQ ID NO:2:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 30 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
Xaa Tyr Gln Ser Ser
1 5
- 40 (2) INFORMATION FOR SEQ ID NO:3:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
45 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 50 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 55

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Xaa Xaa Lys Tyr Gln Ser Ser
1 5

5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

20

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Xaa Lys Tyr Gln Ser Ser
1 5

25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

40

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

(A) NAME/KEY: Other

(B) LOCATION: 3...3

45

(D) OTHER INFORMATION: homoarginine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Xaa Xaa Tyr Gln Ser Ser
1 5

50

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

55

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 5 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
10 a hydrophilic moiety
- (A) NAME/KEY: Other
(B) LOCATION: 3...3
(D) OTHER INFORMATION: homoarginine
15
- (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylalanine
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Xaa Xaa Xaa Xaa Gln Ser Ser
1 5
- 25 (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 35 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
40 a hydrophilic moiety
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- Xaa Xaa Ser Tyr Gln Ser Xaa
1 5
- 45 (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
50 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
55 (ix) FEATURE:

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- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- Xaa Tyr Gln Ser Xaa
1 5
- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
- Xaa Xaa Ser Xaa Gln Ser Xaa
1 5
- (2) INFORMATION FOR SEQ ID NO:10:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- (A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: cyclohexylglycine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Xaa Xaa Gln Ser Xaa
1 5

5 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
20 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa Tyr Gln Ser Ser Ser Leu
1 5

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
40 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Xaa Xaa Ser Tyr Gln Ser Ala
1 5

45 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- 80 -

- (A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: cyclic amino acid substituted with
5 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 Ala Xaa Xaa Ser Tyr Tyr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:14:

- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 20 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 3...3
25 (D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

30 Ala Asn Xaa Xaa Ser Tyr Gln Ser
1 5

(2) INFORMATION FOR SEQ ID NO:15:

- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
40 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
45 (D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

50 Xaa Xaa Ser Tyr Gln Ser Ser Thr
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- 55 (i) SEQUENCE CHARACTERISTICS:

- 81 -

- 5 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 10 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
Xaa Tyr Gln Ser Ser Thr
1 5
- 20 (2) INFORMATION FOR SEQ ID NO:17:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 30 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
Xaa Xaa Ser Tyr Gln Ser Ser Ser
1 5
- 40 (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
45 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 50 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

- 82 -

Xaa Tyr Gln Ser Ser Ser
1 5

5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Xaa Lys Tyr Gln Ser Ser Ser
1 5

25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

40

(A) NAME/KEY: Other

(B) LOCATION: 3...3

(D) OTHER INFORMATION: homoarginine

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Xaa Xaa Xaa Tyr Gln Ser Ser Ser
1 5

50

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

55

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

- 83 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

5 (A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with

10 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Xaa Xaa Ser Tyr Gln Ser Ser Leu

15 1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with

30 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Xaa Tyr Gln Ser Ser Leu

35 1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45 (ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with

50 a hydrophilic moiety

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

55 Xaa Xaa Ser Tyr Gln Ser Leu

1 5

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(2) INFORMATION FOR SEQ ID NO:24:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
(A) NAME/KEY: Other
(B) LOCATION: 1...1
15 (D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
- 20 Xaa Tyr Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:25:

- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
(A) NAME/KEY: Other
35 (B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- (A) NAME/KEY: Other
40 (B) LOCATION: 7...7
(D) OTHER INFORMATION: norleucine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
- 45 Xaa Xaa Ser Tyr Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:26:

- 50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
55 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

5 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

10 (A) NAME/KEY: Other
(B) LOCATION: 5...5
(D) OTHER INFORMATION: norleucine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

15 Xaa Tyr Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:27:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

30 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

35 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-
carboxylic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

40 Xaa Xaa Ser Tyr Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:28:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

55 (A) NAME/KEY: Other
(B) LOCATION: 1...1

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(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

5

(A) NAME/KEY: Other

(B) LOCATION: 5...5

(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-
carboxylic acid

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Xaa Tyr Gln Ser Xaa
1 5

15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

20

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

25

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

30

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Xaa Xaa Ser Xaa Gln Ser Leu
1 5

40

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

45

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

50

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

55

(A) NAME/KEY: Other

(B) LOCATION: 2...2

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(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

5 Xaa Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:31:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20 (A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

25 (A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

30 (A) NAME/KEY: None

(B) LOCATION: 7...7

(D) OTHER INFORMATION: norleucine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

35 Xaa Xaa Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:32:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

50 (A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

55 (A) NAME/KEY: Other

(B) LOCATION: 2...2

(D) OTHER INFORMATION: cyclohexylglycine

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- (A) NAME/KEY: Other
- (B) LOCATION: 5...5
- (D) OTHER INFORMATION: norleucine

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Xaa Xaa Gln Ser Leu
1 5

10 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20 (ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: cyclic amino acid substituted with
25 a hydrophilic moiety

- (A) NAME/KEY: Other
- (B) LOCATION: 4...4
- (D) OTHER INFORMATION: cyclohexylglycine

30 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-
carboxylic acid

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Xaa Xaa Ser Xaa Gln Ser Xaa
1 5

40 (2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- 45 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50 (ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: cyclic amino acid substituted with
55 a hydrophilic moiety

(A) NAME/KEY: Other

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- (B) LOCATION: 2...2
(D) OTHER INFORMATION: cyclohexylglycine
- 5 (A) NAME/KEY: Other
(B) LOCATION: 5...5
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
Xaa Xaa Gln Ser Xaa
1 5
- 15 (2) INFORMATION FOR SEQ ID NO:35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 25 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety
- 30 (A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: homoarginine
- 35 (A) NAME/KEY: Other
(B) LOCATION: 3...3
(D) OTHER INFORMATION: cyclohexylglycine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
40 Xaa Xaa Xaa Gln Ser Leu
1 5
- (2) INFORMATION FOR SEQ ID NO:36:
45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
50 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 55 (A) NAME/KEY: Other
(B) LOCATION: 1...1

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(D) OTHER INFORMATION: cyclic amino acid substituted with
a hydrophilic moiety

5

- (A) NAME/KEY: Other
- (B) LOCATION: 2...2
- (D) OTHER INFORMATION: homoarginine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

10

Xaa Xaa Tyr Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:37:

15

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

20

- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:

25

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

30

Pro Xaa Ser Tyr Gln Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:38:

35

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

40

- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:

45

- (A) NAME/KEY: None
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

50

Pro Xaa Ser Tyr Gln Ser Ala
1 5

(2) INFORMATION FOR SEQ ID NO:39:

55

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids

- 91 -

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 2...2
10 (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

15 Ala Pro Xaa Ser Tyr Tyr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:40:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 3...3
30 (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

35 Ala Asn Pro Xaa Ser Tyr Gln Ser
1 5

(2) INFORMATION FOR SEQ ID NO:41:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...1
50 (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

55 Pro Xaa Ser Tyr Gln Ser Ser Thr
1 5

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(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Pro Tyr Gln Ser Ser Thr
1 5

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Pro Xaa Ser Tyr Gln Ser Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

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Pro Tyr Gln Ser Ser Ser
1 5

5

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Pro Xaa Lys Tyr Gln Ser Ser Ser
1 5

25

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

40

- (A) NAME/KEY: Other
(B) LOCATION: 3...3
(D) OTHER INFORMATION: homoarginine

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Pro Xaa Xaa Tyr Gln Ser Ser Ser
1 5

50

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

5

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

10

Pro Xaa Ser Tyr Gln Ser Ser Leu

1

5

(2) INFORMATION FOR SEQ ID NO:48:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

25

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

30

Pro Tyr Gln Ser Ser Leu

1

5

(2) INFORMATION FOR SEQ ID NO:49:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

45

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

50

Pro Xaa Ser Tyr Gln Ser Leu

1

5

(2) INFORMATION FOR SEQ ID NO:50:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

- 95 -

- (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 5 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
10 (D) OTHER INFORMATION: 4-hydroxyproline
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
- 15 Pro Tyr Gln Ser Leu
1 5
- (2) INFORMATION FOR SEQ ID NO:51:
- 20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
30 (D) OTHER INFORMATION: 4-hydroxyproline
- (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: norleucine
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
- Pro Xaa Ser Tyr Gln Ser Leu
1 5
- 40 (2) INFORMATION FOR SEQ ID NO:52:
- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 50 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline
- 55 (A) NAME/KEY: Other

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- (B) LOCATION: 5...5
(D) OTHER INFORMATION: norleucine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

5

Pro Tyr Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:53:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

25

- (A) NAME/KEY: Other
(B) LOCATION: 7...7

(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

30

Pro Xaa Ser Tyr Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:54:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

45

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

50

- (A) NAME/KEY: Other
(B) LOCATION: 5...5

(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

55

Pro Tyr Gln Ser Xaa

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1

5

(2) INFORMATION FOR SEQ ID NO:55:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

15

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

20

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

25

Pro Xaa Ser Xaa Gln Ser Leu

1

5

(2) INFORMATION FOR SEQ ID NO:56:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

40

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

45

(A) NAME/KEY: Other

(B) LOCATION: 2...2

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

50

Pro Xaa Gln Ser Leu

1

5

(2) INFORMATION FOR SEQ ID NO:57:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

- 98 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

5 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

10 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

15 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: norleucine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

20 Pro Xaa Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:58:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

35 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline

40 (A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: cyclohexylglycine

45 (A) NAME/KEY: Other
(B) LOCATION: 5...5
(D) OTHER INFORMATION: norleucine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Xaa Gln Ser Leu
1 5

50 (2) INFORMATION FOR SEQ ID NO:59:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

- 99 -

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
- 5 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline
- 10 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine
- 15 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:
- 20 Pro Xaa Ser Xaa Gln Ser Xaa
1 5
- (2) INFORMATION FOR SEQ ID NO:60:
- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
- 35 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: 4-hydroxyproline
- 40 (A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: cyclohexylglycine
- 45 (A) NAME/KEY: Other
(B) LOCATION: 5...5
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:
- 50 Pro Xaa Gln Ser Xaa
1 5
- (2) INFORMATION FOR SEQ ID NO:61:
- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids

- 100 -

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

10 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: homoarginine

15 (A) NAME/KEY: Other
(B) LOCATION: 3...3
(D) OTHER INFORMATION: 4-hydroxyproline

(A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Xaa Ser Pro Xaa Gln Ser Leu
1 5

25 (2) INFORMATION FOR SEQ ID NO:62:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 2...2
(D) OTHER INFORMATION: 4-hydroxyproline

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Asn Pro Ile Ser Tyr Gln Ser
1 5

45 (2) INFORMATION FOR SEQ ID NO:63:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

55 (A) NAME/KEY: Other

- 101 -

(B) LOCATION: 2...2

(D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

5

Asn Pro Val Ser Tyr Gln Ser
1 5

(2) INFORMATION FOR SEQ ID NO:64:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

25

Pro Ala Ser Tyr Gln Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:65:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

40

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 3,4-dihydroxyproline

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

45

Xaa Ala Ser Tyr Gln Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:66:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

5

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 3-hydroxyproline

10

(A) NAME/KEY: Other

(B) LOCATION: 3...3

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

15

Xaa Ser Xaa Gln Ser

1

5

(2) INFORMATION FOR SEQ ID NO:67:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

30

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

35

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

40

Pro Ala Ser Xaa Gln Ser Ser

1

5

(2) INFORMATION FOR SEQ ID NO:68:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

55

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-acetyl-4-hydroxyproline

(A) NAME/KEY: Other

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(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

5

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:69:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline

25

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

30

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:70:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

45

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: 4-hydroxyproline

50

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

55

Pro Ala Ser Xaa Gln Ser Leu
1 5

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(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-glutaryl-4-hydroxyproline

- (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-acetyl-3,4-dihydroxyproline

- (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

5 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-(PEG-2)-4-hydroxyproline

10 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

15 Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:74:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

30 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-acetyl-4-hydroxyproline

35 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: 2-amino-4,4-dimethylpropanoic acid

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Xaa Ala Ser Xaa Gln Ser Xaa
1 5

45 (2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

55 (A) NAME/KEY: Other

- 106 -

(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline

5 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

10 Xaa Ala Ser Xaa Gln Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:76:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

25 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-glutaryl-4-hydroxyproline

(A) NAME/KEY: Other
30 (B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

35 Xaa Ala Ser Xaa Gln Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:77:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
50 (B) LOCATION: 1...1
(D) OTHER INFORMATION: N-glutaryl-4-hydroxyproline

(A) NAME/KEY: Other
(B) LOCATION: 4...4
55 (D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

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Xaa Ala Ser Xaa Gln Ser Ile
1 5

5 (2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline

- 20 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Xaa Ser Ser Xaa Gln Ser Ile
1 5

30 (2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40 (ix) FEATURE:

- (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline

- 45 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

50 Xaa Ala Ser Xaa Gln Ser Ile
1 5

(2) INFORMATION FOR SEQ ID NO:80:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids

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- (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 5 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 10 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline
- 15 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine
- (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: 2-aminobutyric acid
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:
- Xaa Ala Ser Xaa Gln Ser Xaa
1 5
- 25 (2) INFORMATION FOR SEQ ID NO:81:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(ix) FEATURE:
- 35 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-glutaryl-4-hydroxyproline
- 40 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:
- 45 Xaa Ser Ser Xaa Gln Ser Ile
1 5
- (2) INFORMATION FOR SEQ ID NO:82:
- 50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
55 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

5 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-glutaryl-4-hydroxyproline

10 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

15 Xaa Ser Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:83:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

30 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-(PEG-2)-4-hydroxyproline

(A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

40 Xaa Ser Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:84:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-succinyl-4-hydroxyproline

55 (A) NAME/KEY: Other

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(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

5 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: thienylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

10 Xaa Ala Ser Xaa Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:85:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

25 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-(PEG-4)-4-hydroxyproline

(A) NAME/KEY: Other
30 (B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

35 Xaa Ser Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:86:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

50 (A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-(PEG-4)-4-hydroxyproline

(A) NAME/KEY: Other
55 (B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

(A) NAME/KEY: Other

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(B) LOCATION: 7...7

(D) OTHER INFORMATION: thienylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

5

Xaa Ala Ser Xaa Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:87:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-succinyl-3,4-dihydroxyproline

25

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

30

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:88:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

40

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

45

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-malonyl-4-hydroxyproline

50

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

55

Xaa Ala Ser Xaa Gln Ser Leu
1 5

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(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: N-acetylproline

- (A) NAME/KEY: Other
- (B) LOCATION: 4...4
- (D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Xaa Ser Ser Xaa Gln Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: N-acetylproline

- (A) NAME/KEY: Other
- (B) LOCATION: 4...4
- (D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-acetyl-3,4-dehydroproline

(A) NAME/KEY: Other

(B) LOCATION: 4...4

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Xaa Ala Ser Xaa Gln Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-acetyl-4-hydroxyproline

(A) NAME/KEY: Other

(B) LOCATION: 2...2

(D) OTHER INFORMATION: cyclohexylglycine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Xaa Xaa Gln Ser Ser Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...1

(D) OTHER INFORMATION: N-acetyl-4-hydroxyproline

(A) NAME/KEY: Other

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(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

5

(A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: thienylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

10

Xaa Ala Ser Xaa Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:94:

15

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

25

(A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-acetyl-4-hydroxyproline

30

(A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

35

carboxylic acid
(D) OTHER INFORMATION: 1,2,3,4-tetrahydroisoquinoline 3-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

40

Xaa Ala Ser Xaa Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:95:

45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

55

(A) NAME/KEY: Other
(B) LOCATION: 1...1
(D) OTHER INFORMATION: N-(hydroxyacetyl)-4-hydroxyproline

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- (A) NAME/KEY: Other
- (B) LOCATION: 4...4
- (D) OTHER INFORMATION: cyclohexylglycine

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Xaa Ala Ser Xaa Gln Ser Val
1 5

10 (2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20 (ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...1
- (D) OTHER INFORMATION: 4-hydroxyproline

25 (A) NAME/KEY: Other
(B) LOCATION: 4...4
(D) OTHER INFORMATION: cyclohexylglycine

30 (A) NAME/KEY: Other
(B) LOCATION: 7...7
(D) OTHER INFORMATION: leucinamide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

35 Pro Ala Ser Xaa Gln Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:97:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- 45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- 50 (B) LOCATION: 1...1
- (D) OTHER INFORMATION: N-(hydroxyacetyl)-4-hydroxyproline

(A) NAME/KEY: Other
(B) LOCATION: 4...4
55 (D) OTHER INFORMATION: cyclohexylglycine

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Xaa Ala Ser Xaa Gln Ser Leu
1 5

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WHAT IS CLAIMED IS:

1. A conjugate which is useful for the treatment of prostate cancer which comprises a cytotoxic agent attached to an oligopeptide, wherein the oligopeptide comprises a sequence of amino acids that is selectively proteolytically cleaved by free prostate specific antigen and wherein the means of attachment is a covalent bond or through a chemical linker, said sequence of amino acids which comprises at least one cyclic amino acid having a hydrophilic substituent;

or the pharmaceutically acceptable salt thereof.

2. The conjugate according to Claim 1 wherein the cytotoxic agent is a member of a class of cytotoxic agents selected from the following classes:

- a) anthracycline family of drugs,
- b) the vinca alkaloid drugs,
- c) the mitomycins,
- d) the bleomycins,
- e) the cytotoxic nucleosides,
- f) the pteridine family of drugs,
- g) diynenes,
- h) estramustine,
- i) cyclophosphamide,
- j) the taxanes and
- k) the podophyllotoxins,

or the pharmaceutically acceptable salt thereof.

3. The conjugate according to Claim wherein the cytotoxic agent is selected from the following cytotoxic agents:

- a) doxorubicin,
- b) carminomycin,

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- c) daunorubicin,
 - d) aminopterin,
 - e) methotrexate,
 - f) methopterin,
 - 5 g) dichloro-methotrexate,
 - h) mitomycin C,
 - i) porfiromycin,
 - j) 5-fluorouracil,
 - k) 6-mercaptopurine,
 - 10 l) cytosine arabinoside,
 - m) podophyllotoxin,
 - n) etoposide,
 - o) etoposide phosphate,
 - p) melphalan,
 - 15 q) vinblastine,
 - r) vincristine,
 - s) leurosidine,
 - t) vindesine,
 - u) estramustine,
 - 20 v) cisplatin,
 - w) cyclophosphamide,
 - x) taxol, and
 - y) leurosine,
- 25 or the pharmaceutically acceptable salt thereof.

4. The conjugate according to Claim 2 wherein the cytotoxic agent is selected from doxorubicin and vinblastine or a cytotoxic derivative thereof.

30

5. The conjugate according to Claim 2 wherein the cytotoxic agent is doxorubicin or a cytotoxic derivative thereof.

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6. The conjugate according to Claim 1 wherein the oligopeptide comprises an oligomer selected from:

- 5 a) HaaXaaSerTyrGlnSerSer (SEQ.ID.NO.: 1);
- b) HaaTyrGlnSerSer (SEQ.ID.NO.: 2);
- c) HaaXaaLysTyrGlnSerSer (SEQ.ID.NO.: 3);
- 10 d) HaaXaaLysTyrGlnSerSer (SEQ.ID.NO.: 4);
- e) HaaXaahArgTyrGlnSerSer (SEQ.ID.NO.: 5);
- f) HaaXaahArgChaGlnSerSer (SEQ.ID.NO.: 6);
- 15 g) HaaXaaSerTyrGlnSerXaa (SEQ.ID.NO.: 7);
- h) HaaTyrGlnSerXaa (SEQ.ID.NO.: 8);
- 20 i) HaaXaaSerChgGlnSerXaa (SEQ.ID.NO.: 9);
- j) HaaChgGlnSerXaa (SEQ.ID.NO.: 10);

25 wherein Haa is a cyclic amino acid substituted with a hydrophilic moiety, Xaa is any amino acid, hArg is homoarginine, Cha is cyclohexylalanine and Chg is cyclohexylglycine.

7. The conjugate according to Claim 1 wherein the oligopeptide comprises an oligomer selected from:

- 30 a) HaaXaaSerTyrGlnSerSer (SEQ.ID.NO.: 11),
- b) HaaXaaSerTyrGlnSerAla (SEQ.ID.NO.: 12),

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- c) AlaHaaXaaSerTyrTyrI Ser (SEQ.ID.NO.: 13),
- d) AlaAsnHaaXaaSerTyrGlnI Ser (SEQ.ID.NO.: 14),
- 5 e) HaaXaaSerTyrGlnI SerSerThr (SEQ.ID.NO.: 15),
- f) HaaTyrGlnI SerSerThr (SEQ.ID.NO.: 16),
- 10 g) HaaXaaSerTyrGlnI SerSerSer (SEQ.ID.NO.: 17),
- h) HaaTyrGlnI SerSerSer (SEQ.ID.NO.: 18),
- i) HaaXaaLysTyrGlnI SerSerSer (SEQ.ID.NO.: 19),
- 15 j) HaaXaaHArgTyrGlnI SerSerSer (SEQ.ID.NO.: 20),
- k) HaaXaaSerTyrGlnI SerSerLeu (SEQ.ID.NO.: 21);
- 20 l) HaaTyrGlnI SerSerLeu (SEQ.ID.NO.: 22);
- m) HaaXaaSerTyrGlnI SerLeu (SEQ.ID.NO.: 23);
- n) HaaTyrGlnI SerLeu (SEQ.ID.NO.: 24);
- 25 p) HaaXaaSerTyrGlnI SerNle (SEQ.ID.NO.: 25);
- q) HaaTyrGlnI SerNle (SEQ.ID.NO.: 26);
- r) HaaXaaSerTyrGlnI SerTIC (SEQ.ID.NO.: 27);
- 30 s) HaaTyrGlnI SerTIC (SEQ.ID.NO.: 28);
- t) HaaXaaSerChgGlnI SerLeu (SEQ.ID.NO.: 29);

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- u) HaaChgGlnSerLeu (SEQ.ID.NO.: 30);
- v) HaaXaaSerChgGlnSerNle (SEQ.ID.NO.: 31);
- 5 w) HaaChgGlnSerNle (SEQ.ID.NO.: 32);
- x) HaaXaaSerChgGlnSerTIC (SEQ.ID.NO.: 33);
- y) HaaChgGlnSerTIC (SEQ.ID.NO.: 34);
- 10 z) hArgChgGlnSerLeu (SEQ.ID.NO.: 35); and
- aa) hArgTyrGlnSerLeu (SEQ.ID.NO.: 36).
- 15 8. The conjugate according to Claim 1 wherein the oligopeptide comprises an oligomer selected from:
- a) 4-HypXaaSerTyrGlnSerSer (SEQ.ID.NO.: 37),
- 20 b) 4-HypXaaSerTyrGlnSerAla (SEQ.ID.NO.: 38),
- c) Ala4-HypXaaSerTyrTyrSer (SEQ.ID.NO.: 39),
- d) AlaAsn4-HypXaaSerTyrGlnSer (SEQ.ID.NO.: 40),
- 25 e) 4-HypXaaSerTyrGlnSerSerThr (SEQ.ID.NO.: 41),
- f) 4-HypTyrGlnSerSerThr (SEQ.ID.NO.: 42),
- 30 g) 4-HypXaaSerTyrGlnSerSerSer (SEQ.ID.NO.: 43),
- h) 4-HypTyrGlnSerSerSer (SEQ.ID.NO.: 44),
- i) 4-HypXaaLysTyrGlnSerSerSer (SEQ.ID.NO.: 45),

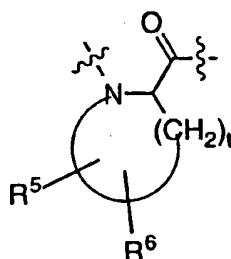
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- j) 4-HypXaahArgTyrGlnSerSerSer (SEQ.ID.NO.: 46),
k) 4-HypXaaSerTyrGlnSerSerLeu (SEQ.ID.NO.: 47);
5 l) 4-HypTyrGlnSerSerLeu (SEQ.ID.NO.: 48);
m) 4-HypXaaSerTyrGlnSerLeu (SEQ.ID.NO.: 49);
10 n) 4-HypTyrGlnSerLeu (SEQ.ID.NO.: 50);
p) 4-HypXaaSerTyrGlnSerNle (SEQ.ID.NO.: 51);
q) 4-HypTyrGlnSerNle (SEQ.ID.NO.: 52);
15 r) 4-HypXaaSerTyrGlnSerTIC (SEQ.ID.NO.: 53);
s) 4-HypTyrGlnSerTIC (SEQ.ID.NO.: 54);
20 t) 4-HypXaaSerChgGlnSerLeu (SEQ.ID.NO.: 55);
u) 4-HypChgGlnSerLeu (SEQ.ID.NO.: 56);
v) 4-HypXaaSerChgGlnSerNle (SEQ.ID.NO.: 57);
25 w) 4-HypChgGlnSerNle (SEQ.ID.NO.: 58);
x) 4-HypXaaSerChgGlnSerTIC (SEQ.ID.NO.: 59);
30 y) 4-HypChgGlnSerTIC (SEQ.ID.NO.: 60);

wherein 4-Hyp is 4-hydroxyproline, Xaa is any amino acid, hArg is homoarginine, Cha is cyclohexylalanine and Chg is cyclohexylglycine.

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9. The conjugate according to Claim 1 wherein the cyclic amino acid having a hydrophilic substituent is selected from:



5 wherein:

R^5 is selected from HO- and C1-C6 alkoxy;

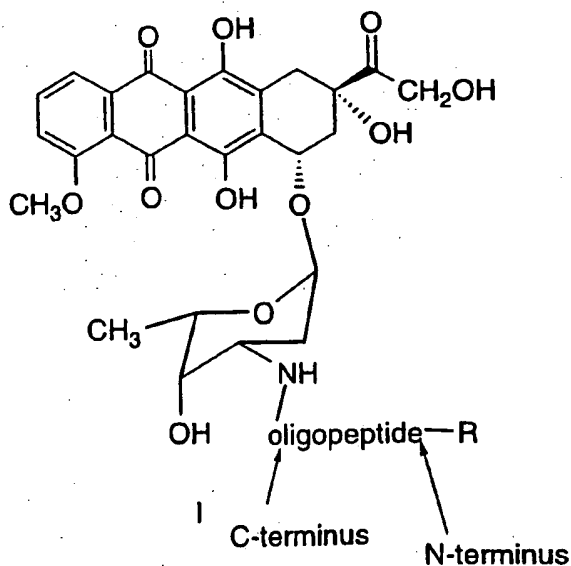
R^6 is selected from hydrogen, halogen, C1-C6 alkyl, HO- and C1-C6 alkoxy; and

10

t is 3 or 4.

10. A conjugate which is useful for the treatment of prostate cancer of the formula I:

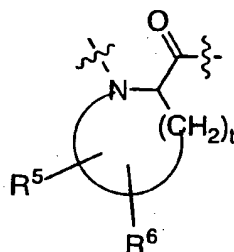
15



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wherein:

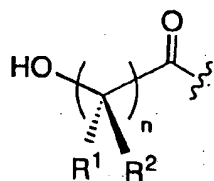
- oligopeptide is an oligopeptide which is selectively recognized by the
 5 free prostate specific antigen (PSA) and is capable of being proteolytically
 cleaved by the enzymatic activity of the free prostate specific
 antigen, wherein the oligopeptide comprises a cyclic amino acid of the
 formula:



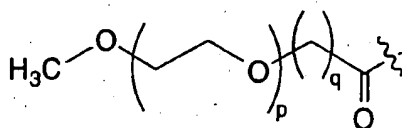
- 10 and wherein the C-terminus carbonyl is covalently bound to the amine
 of doxorubicin;

R is selected from

- 15 a) hydrogen,
 b) $-(C=O)R^1a$,
 c)



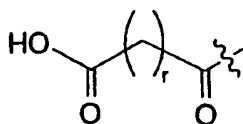
d)



20

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e)



5 R^1 and R^2 are independently selected from: hydrogen, OH, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 aralkyl and aryl;

R^{1a} is C1-C6-alkyl, hydroxylated aryl, polyhydroxylated aryl or aryl,

10 R^5 is selected from HO- and C1-C6 alkoxy;

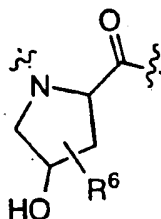
R^6 is selected from hydrogen, halogen, C1-C6 alkyl, HO- and C1-C6 alkoxy; and

15 n is 1, 2, 3 or 4;
 p is zero or an integer between 1 and 100;
 q is 0 or 1, provided that if p is zero, q is 1;
 r is an integer between 1 and 10; and
 t is 3 or 4;

20 or a pharmaceutically acceptable salt thereof.

11. The conjugate according to Claim 10 wherein:

the cyclic amino acid is

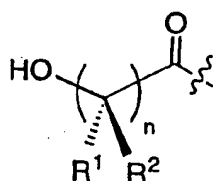


25

R is selected from

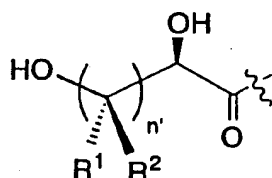
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- a) hydrogen,
 b) $-(C=O)R^{1a}$,
 c)

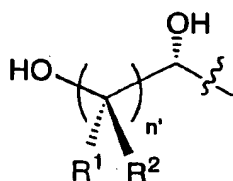


5

- d)

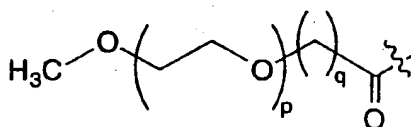


- e)



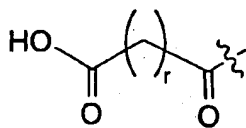
10

- f)



15

- g)



R¹ and R² are independently selected from: hydrogen, C₁-C₆ alkyl and aryl;

20

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R^{1a} is C₁-C₆-alkyl or aryl,

- n is 1, 2, 3 or 4;
5 n' is 0, 1, 2 or 3;
p is zero or an integer between 1 and 14;
q is 0 or 1, provided that if p is zero, q is 1;
r is an integer between 1 and 10;
t is 3;

10

or a optical isomer or pharmaceutically acceptable salt thereof.

12. The conjugate according to Claim 10 wherein:
oligopeptide is an oligomer that comprises an amino acid sequence
15 selected from:

- a) 4-HypXaaSerTyrGlnSerSer (SEQ.ID.NO.: 37),
b) 4-HypXaaSerTyrGlnSerAla (SEQ.ID.NO.: 38),
20 c) Ala-4-HypXaaSerTyrTyrSer (SEQ.ID.NO.: 39),
d) AlaAsn4-HypXaaSerTyrGlnSer (SEQ.ID.NO.: 40),
25 e) 4-HypXaaSerTyrGlnSerSerThr (SEQ.ID.NO.: 41),
f) 4-HypTyrGlnSerSerThr (SEQ.ID.NO.: 42),
g) 4-HypXaaSerTyrGlnSerSerSer (SEQ.ID.NO.: 43),
30 h) 4-HypTyrGlnSerSerSer (SEQ.ID.NO.: 44),
i) 4-HypXaaLysTyrGlnSerSerSer (SEQ.ID.NO.: 45),

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- j) 4-HypXaahArgTyrGlnSerSerSer (SEQ.ID.NO.: 46),
k) 4-HypXaaSerTyrGlnSerSerLeu (SEQ.ID.NO.: 47);
5 l) 4-HypTyrGlnSerSerLeu (SEQ.ID.NO.: 48);
m) 4-HypXaaSerTyrGlnSerLeu (SEQ.ID.NO.: 49);
n) 4-HypTyrGlnSerLeu (SEQ.ID.NO.: 50);
10 p) 4-HypXaaSerTyrGlnSerNle (SEQ.ID.NO.: 51);
q) 4-HypTyrGlnSerNle (SEQ.ID.NO.: 52);
15 r) 4-HypXaaSerTyrGlnSerTIC (SEQ.ID.NO.: 53);
s) 4-HypTyrGlnSerTIC (SEQ.ID.NO.: 54);
t) 4-HypXaaSerChgGlnSerLeu (SEQ.ID.NO.: 55);
20 u) 4-HypChgGlnSerLeu (SEQ.ID.NO.: 56);
v) 4-HypXaaSerChgGlnSerNle (SEQ.ID.NO.: 57);
25 w) 4-HypChgGlnSerNle (SEQ.ID.NO.: 58);
x) 4-HypXaaSerChgGlnSerTIC (SEQ.ID.NO.: 59);
y) 4-HypChgGlnSerTIC (SEQ.ID.NO.: 60);
30

wherein 4-Hyp is 4-hydroxyproline, Xaa is any amino acid, hArg is homoarginine, Cha is cyclohexylalanine and Chg is cyclohexylglycine;

or an optical isomer or pharmaceutically acceptable salt thereof.

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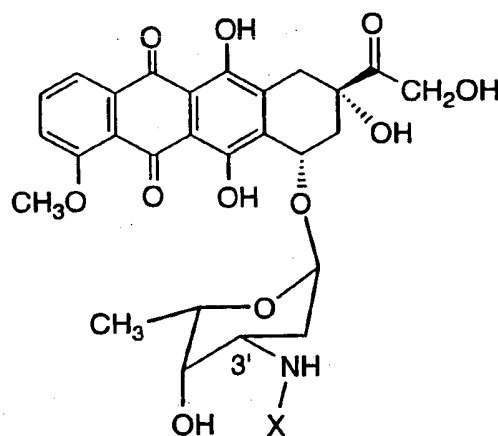
13. The conjugate according to Claim 12 wherein:

Xaa is alanine or isoleucine;

5

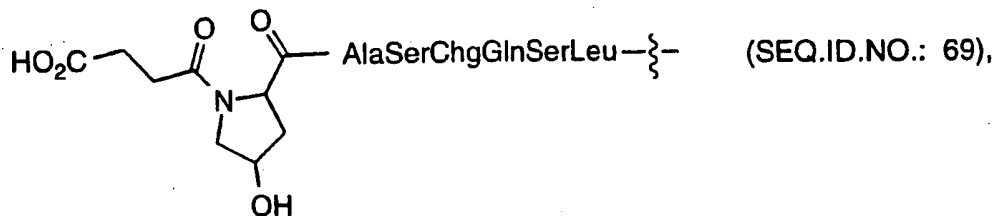
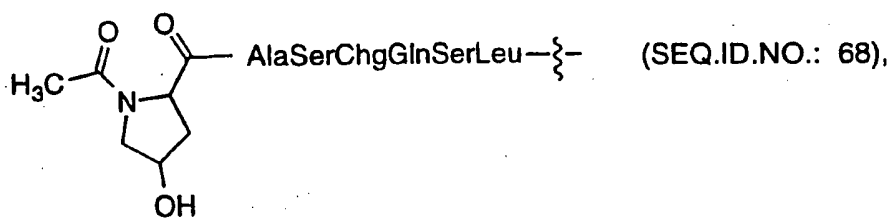
or an optical isomer or pharmaceutically acceptable salt thereof.

14. The conjugate according to Claim 10 which is selected from:

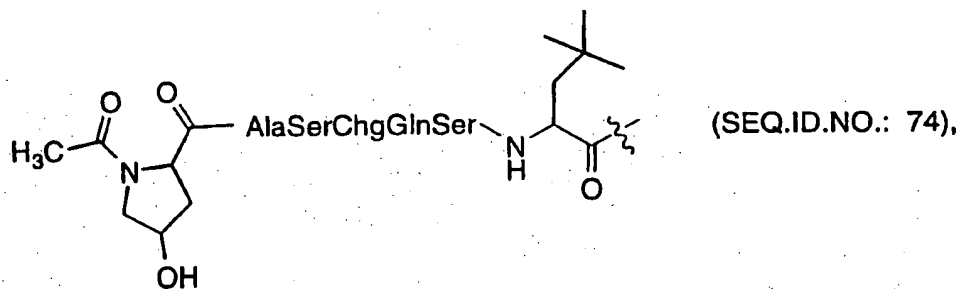
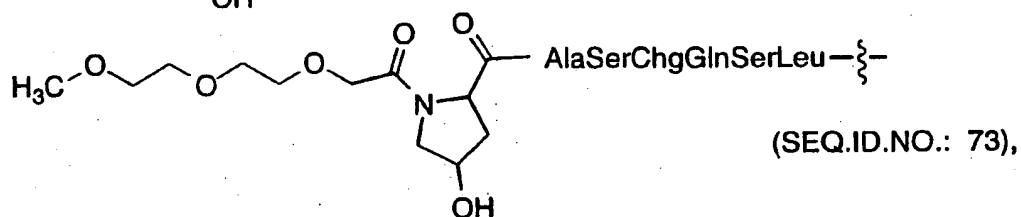
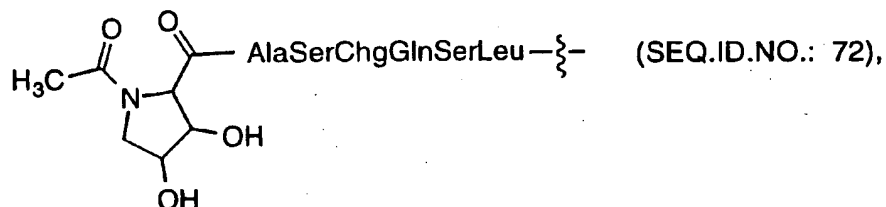
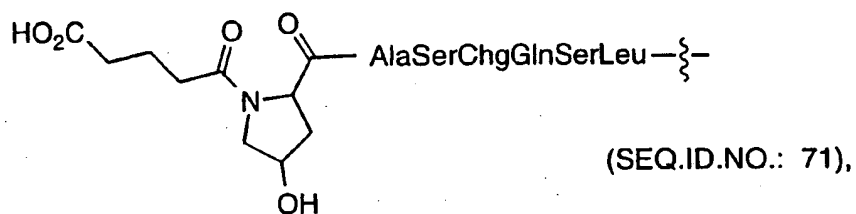
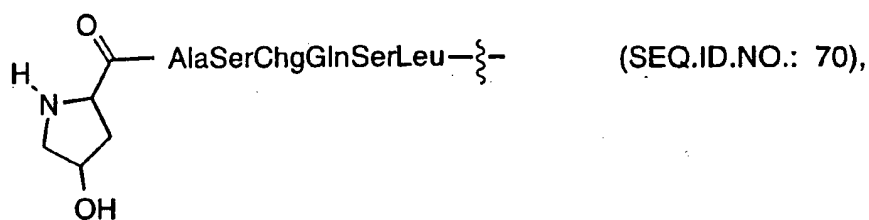


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wherein X is:



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	SEQ. ID. NO.
Succinyl-(4-Hyp)ASChgQ-SV-DOX (3')	75
Glutaryl-(4-Hyp)ASChgQ-SV-DOX (3')	76
Glutaryl-(4-Hyp)ASChgQ-SI-DOX (3')	77
Succinyl-(4-Hyp)SSChgQ-SI-DOX (3')	78

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Succinyl-(4-Hyp)ASChgQ-SI-DOX (3')	79
Succinyl-(4-Hyp)ASChgQ-SAbu-DOX (3')	80
Glutaryl-(4-Hyp)SSChgQ-SI-DOX (3')	81
Glutaryl-(4-Hyp)SSChgQ-SL-DOX (3')	82
PEG(2)-(4-Hyp)SSChgQ-SL-DOX (3')	83
Succinyl-(4-Hyp)ASChgQ-SThi-DOX (3')	84
PEG(4)-(4-Hyp)-SSChgQ-SL-DOX (3')	85
PEG(2)-(4-Hyp)ASChgQ-SThi-DOX(3')	86
Succinyl-3,4-(diOH)PASChgQ-SL-DOX (3')	87
Malonyl-(4-Hyp)ASChgQ-SL-DOX (3')	88

or an optical isomer or pharmaceutically acceptable salt thereof.

15. The conjugate according to Claim 10 which is:

5

	SEQ. ID. NO.
Succinyl-(4-trans-L-Hyp)ASChgQ-SV-DOX (3')	75
Glutaryl-(4-trans-L-Hyp)ASChgQ-SV-DOX (3')	76
Glutaryl-(4-trans-L-Hyp)ASChgQ-SI-DOX (3')	77
Succinyl-(4-trans-L-Hyp)SSChgQ-SI-DOX (3')	78
Succinyl-(4-trans-L-Hyp)ASChgQ-SI-DOX (3')	79
Succinyl-(4-trans-L-Hyp)ASChgQ-SAbu-DOX (3')	80
Glutaryl-(4-trans-L-Hyp)SSChgQ-SI-DOX (3')	81
Glutaryl-(4-trans-L-Hyp)SSChgQ-SL-DOX (3')	82
PEG(2)-(4-trans-L-Hyp)SSChgQ-SL-DOX (3')	83
Succinyl-(4-trans-L-Hyp)ASChgQ-SThi-DOX (3')	84
PEG(4)-(4-trans-L-Hyp)-SSChgQ-SL-DOX (3')	85
PEG(2)-(4-trans-L-Hyp)ASChgQ-SThi-DOX(3')	86
Succinyl-3,4-(diOH)PASChgQ-SL-DOX (3')	87
Malonyl-(4-trans-L-Hyp)ASChgQ-SL-DOX (3')	88

or an optical isomer or pharmaceutically acceptable salt thereof.

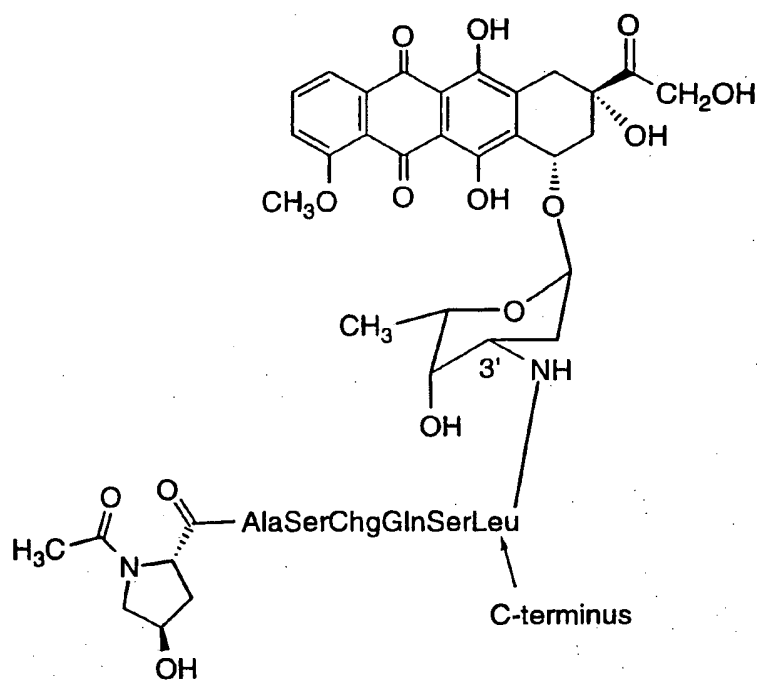
16. The conjugate according to Claim 10 which is:

10

[N-Ac-(4-trans-L-Hyp)]-Ala-Ser-Chg-Gln-Ser-Leu-Dox

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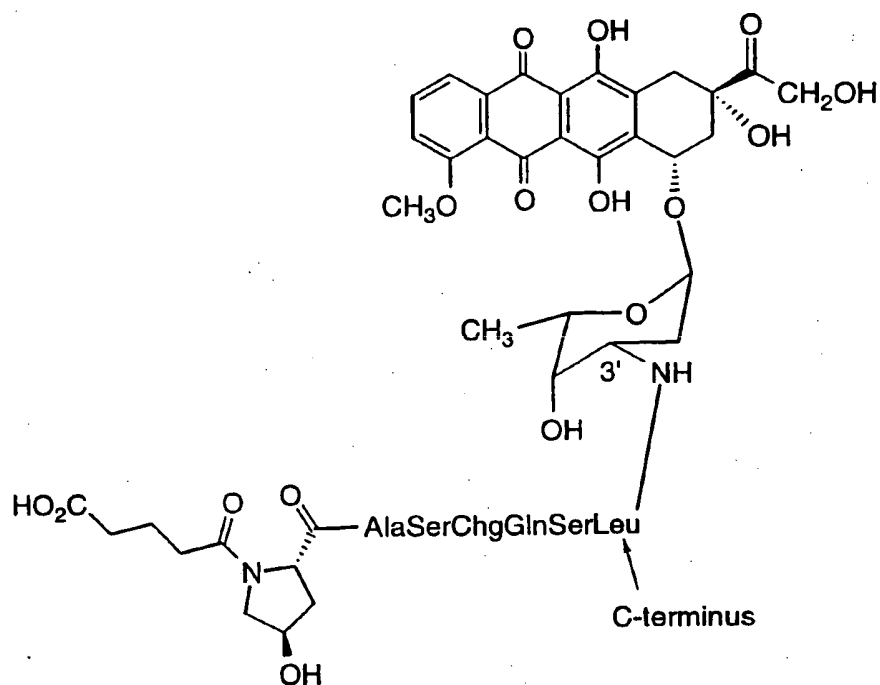
(SEQ.ID.NO.: 68)



or an optical isomer or pharmaceutically acceptable salt thereof.

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17. The conjugate according to Claim 10 which is:

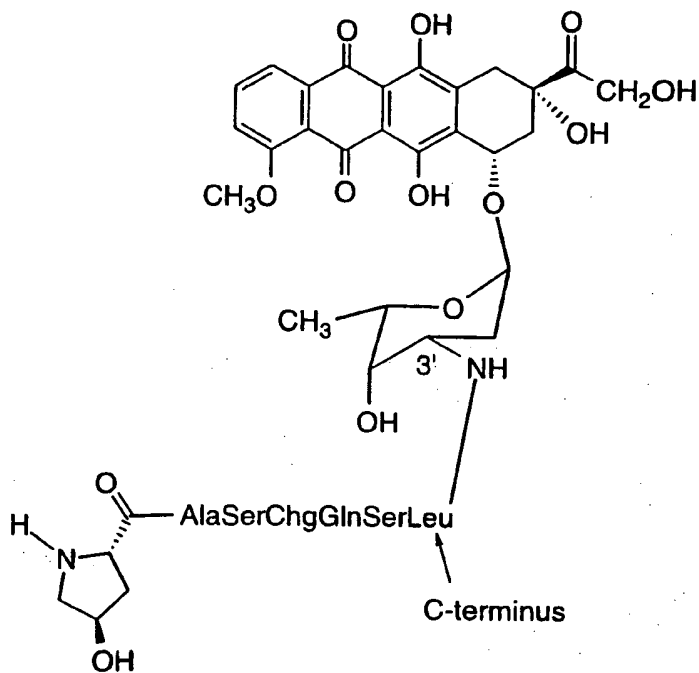


(SEQ.ID.NO.: 71)

or an optical isomer or pharmaceutically acceptable salt thereof.

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18. The conjugate according to Claim 10 which is:

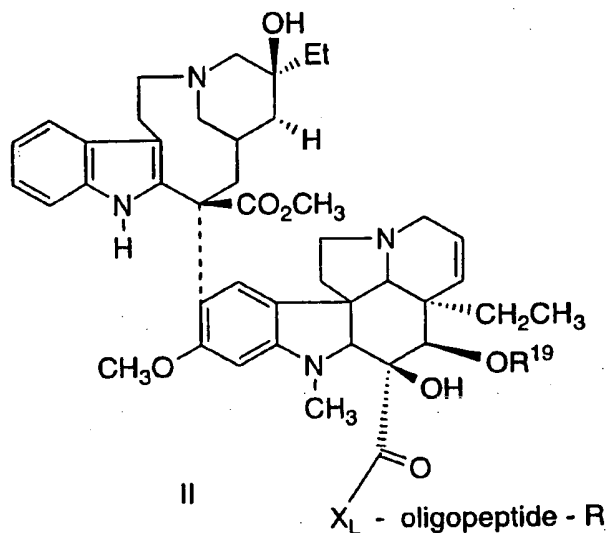


(SEQ.ID.NO.: 70)

5 or an optical isomer or pharmaceutically acceptable salt thereof.

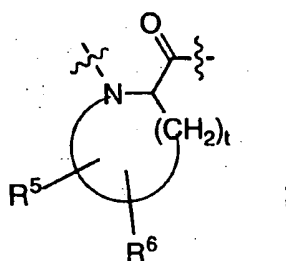
19. The conjugate according to Claim 1 of the formula II:

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wherein:

- 5 oligopeptide is an oligopeptide which is specifically recognized by the free prostate specific antigen (PSA) and is capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen, and the oligopeptide comprises a cyclic amino acid of the formula:



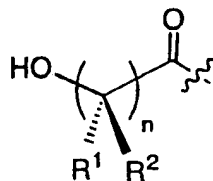
XL is - NH - (CH₂)_u - NH -

R is selected from

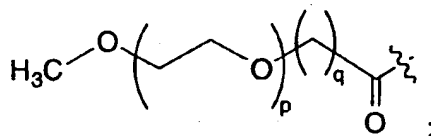
- 15 a) hydrogen,
b) -(C=O)R_{1a},

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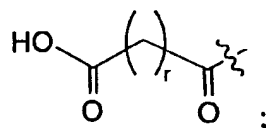
c)



d)



e)



10 R1 and R2 are independently selected from: hydrogen, OH, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 aralkyl and aryl;

R1a is C1-C6-alkyl, hydroxylated aryl, polyhydroxylated aryl or aryl,

15 R19 is hydrogen, (C1-C3 alkyl)-CO, or chlorosubstituted (C1-C3 alkyl)-CO;

n is 1, 2, 3 or 4;

p is zero or an integer between 1 and 100;

q is 0 or 1, provided that if p is zero, q is 1;

20 r is 1, 2 or 3;

t is 3 or 4;

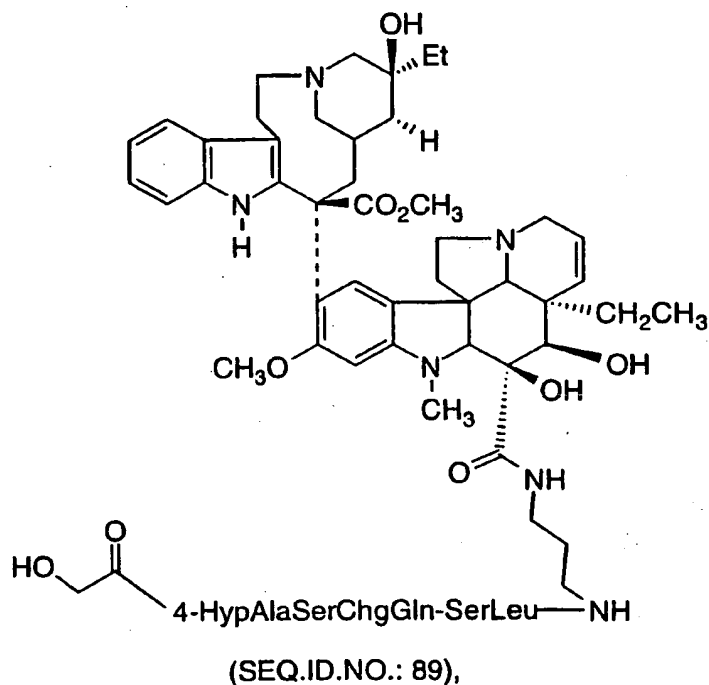
u is 1, 2, 3, 4 or 5,

or a pharmaceutically acceptable salt thereof.

25

20. The conjugate according to Claim 16 which is selected from:

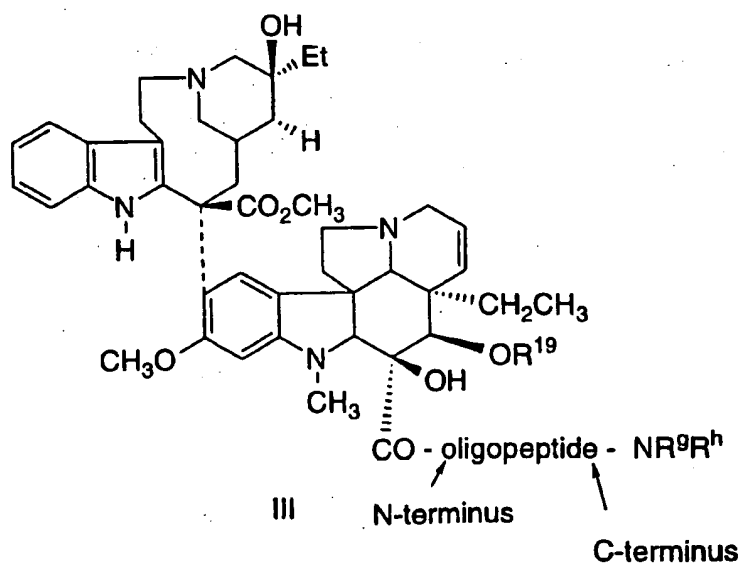
- 137 -



or a pharmaceutically acceptable salt or optical isomer thereof.

21. The conjugate according to Claim 1 of the formula

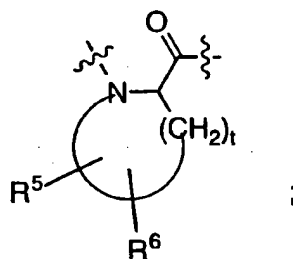
5 III:



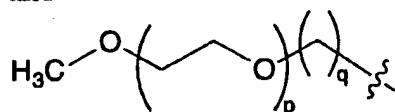
- 138 -

wherein:

- oligopeptide is an oligopeptide which is specifically recognized by the free prostate specific antigen (PSA) and is capable of being proteolytically cleaved by the enzymatic activity of the free prostate specific antigen, and the oligopeptide comprises a cyclic amino acid of the formula:



- R^g and R^h are independently selected from: hydrogen, C₁-C₆-alkyl, -C₁-C₆-alkyl-OH, -C₁-C₆-alkyl-di-OH, -C₁-C₆-alkyl-tri-OH and



provided that at least one R^d and R^e are not hydrogen or C₁-C₆-alkyl, or

- R^g and R^h are combined to form a -CH₂CH₂OCH₂CH₂- diradical;

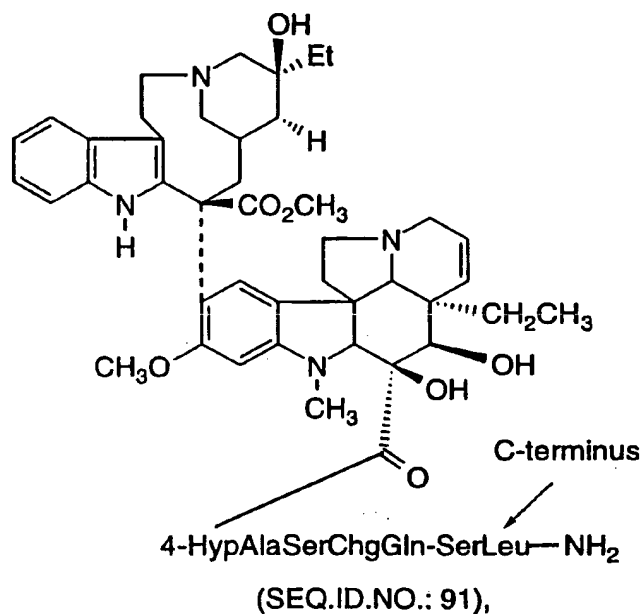
R^{19} is hydrogen, (C₁-C₃ alkyl)-CO, or chloro-substituted (C₁-C₃ alkyl)-CO;

- p is zero or an integer between 1 and 100;
 q is 0 or 1, provided that if p is zero, q is 1;

or a pharmaceutically acceptable salt thereof.

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22. The conjugate according to Claim 18 which is:



or a pharmaceutically acceptable salt or optical isomer thereof.

5 23. A pharmaceutical composition comprising a
pharmaceutical carrier, and dispersed therein, a therapeutically effective
amount of a compound of Claim 1.

10 24. A pharmaceutical composition comprising a
pharmaceutical carrier, and dispersed therein, a therapeutically effective
amount of a compound of Claim 10.

15 25. A pharmaceutical composition comprising a
pharmaceutical carrier, and dispersed therein, a therapeutically effective
amount of a compound of Claim 14.

20 26. A pharmaceutical composition comprising a
pharmaceutical carrier, and dispersed therein, a therapeutically effective
amount of a compound of Claim 17.

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27. A method for treating prostate cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 23.

5 28. A method for treating prostate cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 24.

10 29. A method for treating prostate cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 25.

15 30. A method for treating prostate cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 26.

20 31. A method for treating benign prostatic hyperplasia which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 23.

32. A method for treating benign prostatic hyperplasia which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 24.

25 33. A method for treating benign prostatic hyperplasia which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 25.

30 34. A method for treating benign prostatic hyperplasia which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 26.

35. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

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36. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

5

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/19225		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
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(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(72) Inventors; and (75) Inventors/Applicants (for US only): GARSKY, Victor, M. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). FENG, Dong-Mei [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). DEFEO-JONES, Deborah [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(88) Date of publication of the international search report: 23 July 1998 (23.07.98)	
(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).			
(54) Title: CONJUGATES USEFUL IN THE TREATMENT OF PROSTATE CANCER			
(57) Abstract <p>Chemical conjugates which comprise oligopeptides, having amino acid sequences that are selectively proteolytically cleaved by free prostate specific antigen (PSA) and known cytotoxic agents are disclosed. Such conjugates are useful in the treatment of prostatic cancer and benign prostatic hypertrophy (BPH).</p>			

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INTERNATIONAL SEARCH REPORT

Inter national Application No
PCT/US 97/19225

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 00503 A (MERCK & CO INC ;DEFEO JONES DEBORAH (US); FENG DONG MEI (US); GARS) 11 January 1996 see the whole document see claims 12-19; example 10	1-36
X,P	WO 97 12624 A (DEFEO JONES DEBORAH ;FENG DONG MEI (US); OLIFF ALLEN I (US); GARSK) 10 April 1997 see the whole document see example 15 see claims 12-24	1-36
X,P	WO 97 14416 A (MERCK & CO INC ;DEFEO JONES DEBORAH (US); JONES RAYMOND E (US); OL) 24 April 1997 see the whole document see claims 1,14,17; example 15	1-36
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the international search

13 May 1998

Date of mailing of the international search report

11. 06. 98

Name and mailing address of the ISA

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Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19225

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NICHIFOR M ET AL: "Chemical and enzymatic hydrolysis of dipeptide derivatives of 5-fluorouracil"</p> <p>JOURNAL OF CONTROLLED RELEASE, vol. 3, no. 47, 8 September 1997, page 271-281 XP004086645 see page 271 - page 272; figure 1; table 1 see page 279, column 2 - page 280</p> <p>-----</p>	1-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/19225

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 27-34
are directed to a method of treatment of the human/animal body , the search
has been carried out and based on the alleged effects of the
compound/composition.
2. ☒ Claims Nos.: 1-5,9-11,19,21,23,24,27,28,31,32,35,36
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-5,9-11,19,21,23,24,27,28,31,32,35,36

In view of the large number of compounds, which are defined by the general definition in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, Part B, Chapter III, paragraph 3.6).

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter: onal Application No

PCT/US 97/19225

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9600503 A	11-01-1996	US 5599686 A	04-02-1997
		AU 3092295 A	25-01-1996
		CA 2192957 A	11-01-1996
		CZ 9603810 A	16-04-1997
		EP 0771209 A	07-05-1997
		FI 965225 A	26-02-1997
		HU 76350 A	28-08-1997
		JP 10502619 T	10-03-1998
		NO 965592 A	28-02-1997
		PL 317872 A	28-04-1997
		SK 164096 A	04-06-1997
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WO 9712624 A	10-04-1997	AU 7203496 A	28-04-1997
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WO 9714416 A	24-04-1997	AU 7432196 A	07-05-1997
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